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Bridging the Gap

From Food to Fuel Analysis

Diverse matrices need diverse analytical techniques

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President, Analytical Sciences Community, RSC
Chair, Analytical Chemistry Trust Fund

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Today's presentation

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- Introduction
- Common themes between industries & applications
- Chromatography
- Elemental analysis
- Spectroscopy
- Mass spectrometry
- Sampling & sample preparation
- Summary
- RSC CPD approved training
- RSC prizes

Food to fuel analysis

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Common themes

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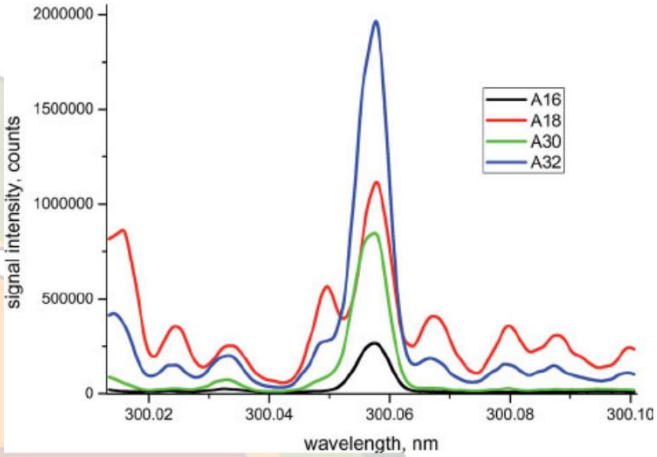
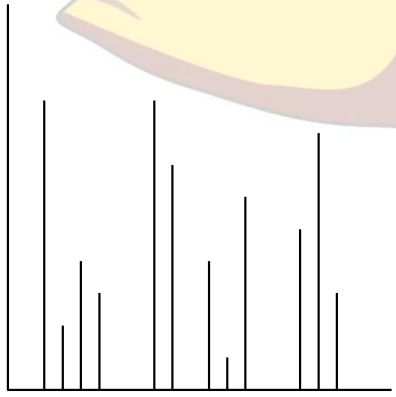
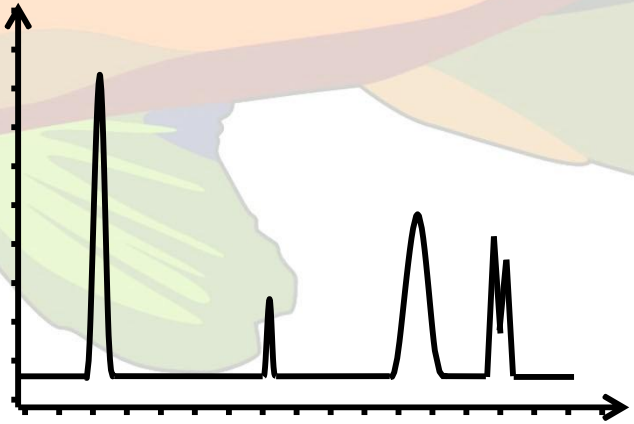
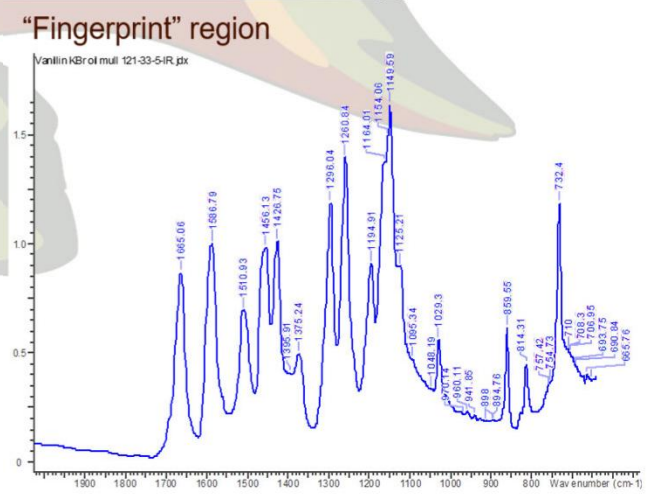
- Sample analysis is used to determine:
  - What components are or aren't present within the sample
  - How much of a component is present
  - Identify unknown components
  - Profiling/biotyping/fingerprinting of the sample









Qualitative data analysis

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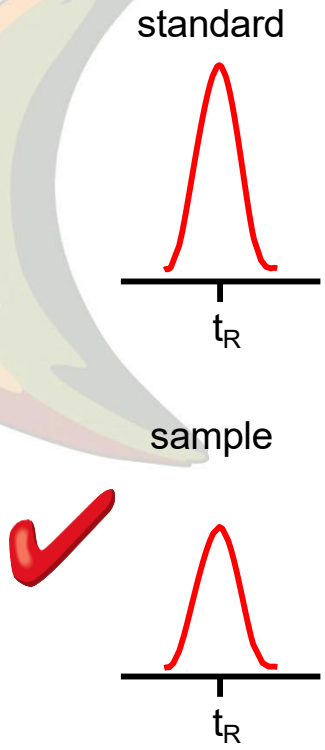
What analytes are in the sample?

- Analysis used to confirm presence or absence of analytes in sample whose identities are known
  - E.g. in chromatography retention time of peak is compared to retention time of a known peak analysed under same conditions
- Analysis may not lead to positive identification of an analyte, but provides evidence of absence of a species (or it is present below detection limit of method)
  - E.g. failure of sample to produce peak at same retention time as standard obtained under identical conditions is strong evidence of absence



Quantitative data analysis

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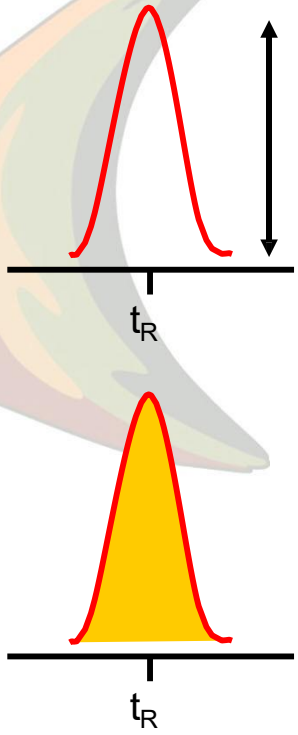
How much of each analyte in sample?

- Compounds must be known (target analytes)
  - E.g. chromatography uses retention time (& mass spectrum) to identify analyte
  - Uses peak height or peak area to quantify how much is there, by comparing to height/area of known concentration of analyte



E.g.: %Ethanol using FTIR

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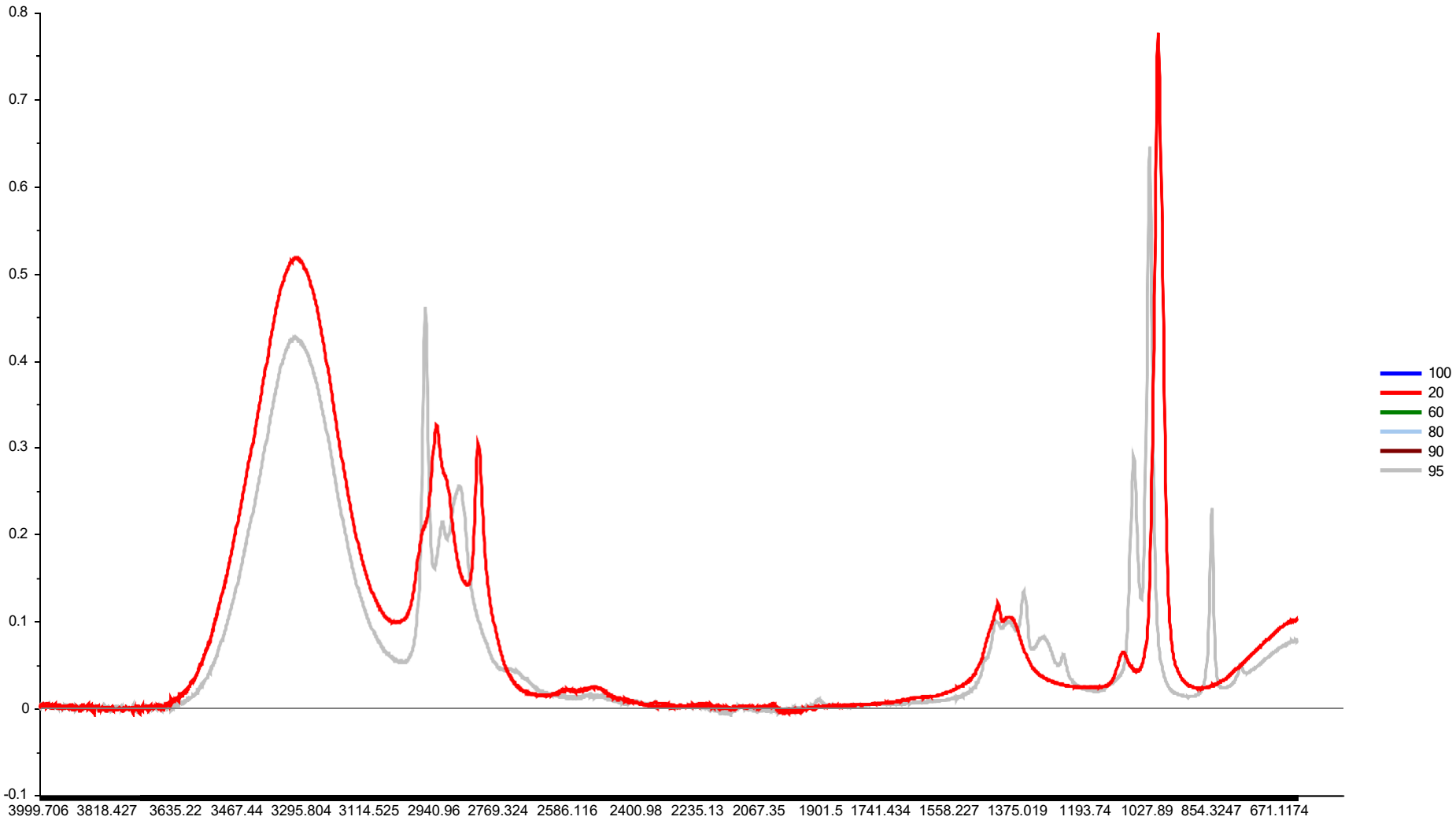

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Identification of unknowns

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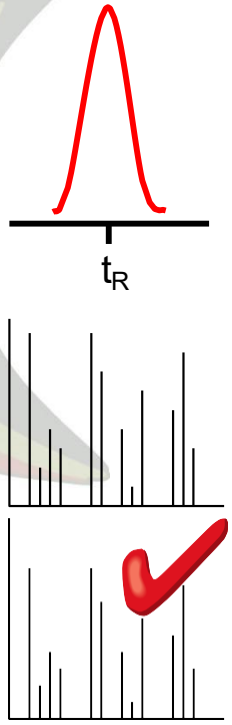
- Identification of unknown analytes in sample needs particular techniques
- E.g. mass spectra obtained with certain MS can be compared to libraries of spectra to identify analyte
- With any MS, mass spectra can be interpreted to determine structure of analyte
- Accurate mass MS enable accurate determination of molecular ion for confirmation



Chromatography

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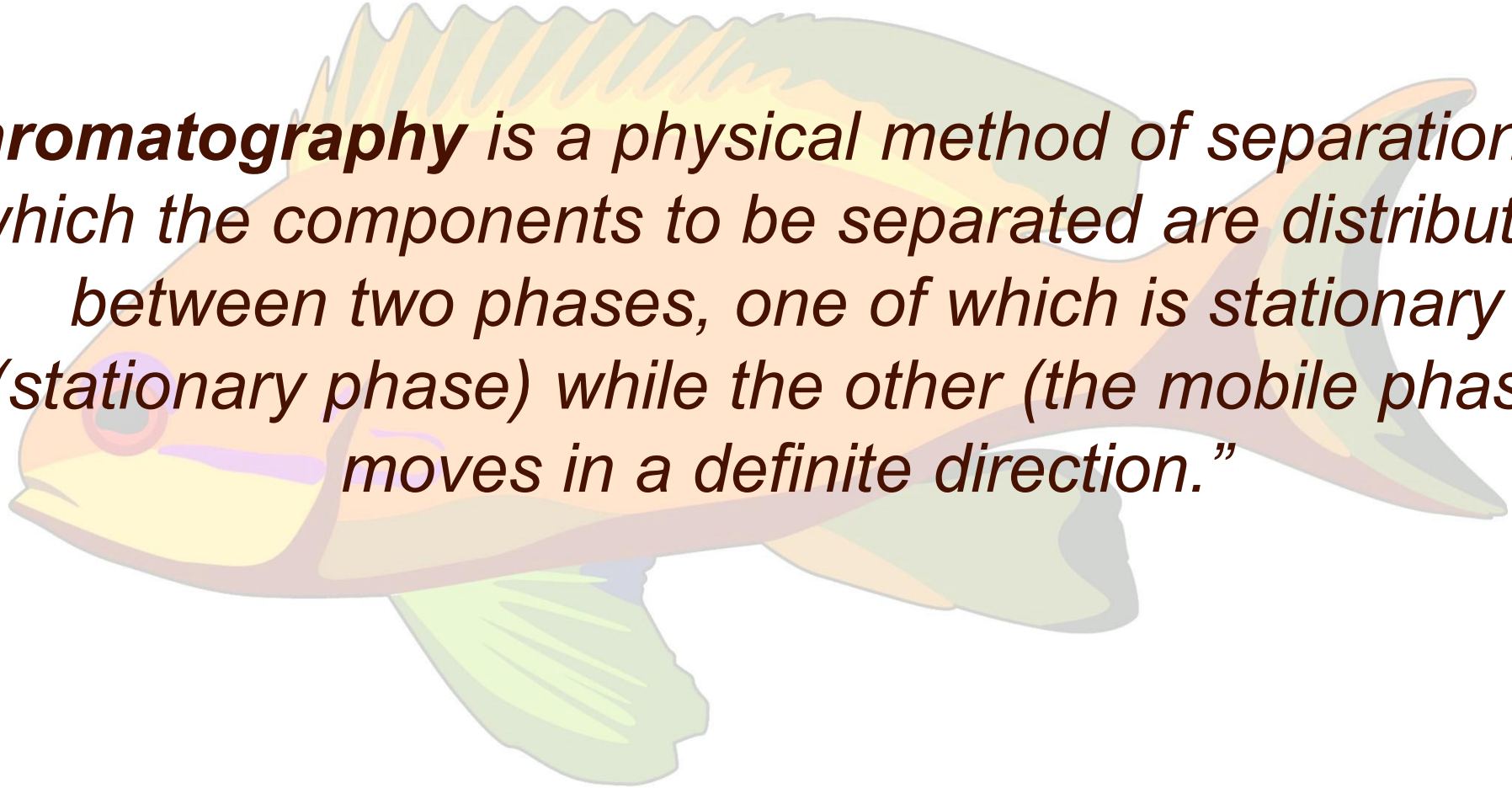

*"**Chromatography** is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction."*

Uses of chromatography

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There are 2 main reasons for performing chromatography

- **Preparative**
  - Aims: separate components of mixture for further use
  - Form of purification
  - Uses large amounts of sample
- **Analytical**
  - Aims: separate components of mixture & identify and/or measure amount of each chemical
  - Identification & quantitation
  - Uses smaller amounts of samples

Types of chromatography

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- Chromatographic bed shape
  - Column chromatography
  - Planar: Paper & Thin layer chromatography (TLC)
- Physical state of mobile phase
  - Gas chromatography (GC)
  - Liquid chromatography (LC)
- Affinity:
  - Supercritical fluid chromatography (SFC)
- Separation mechanism:
  - Ion exchange chromatography (IEC)
  - Size exclusion chromatography (SEC)

Samples

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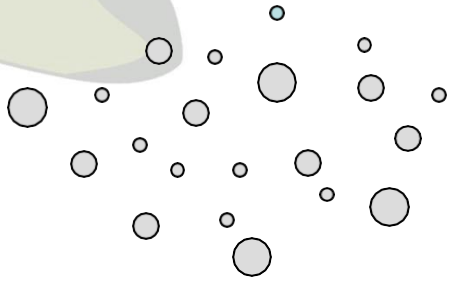
"components to be separated"

- A sample is made up of:
  - Analytes = compounds of interest
  - Matrix = other components not interested in
  - Matrix interference = matrix component(s) which interfere with the analysis of analytes
- Can be a Solid a Liquid or a Gas



Analytes: chromatography

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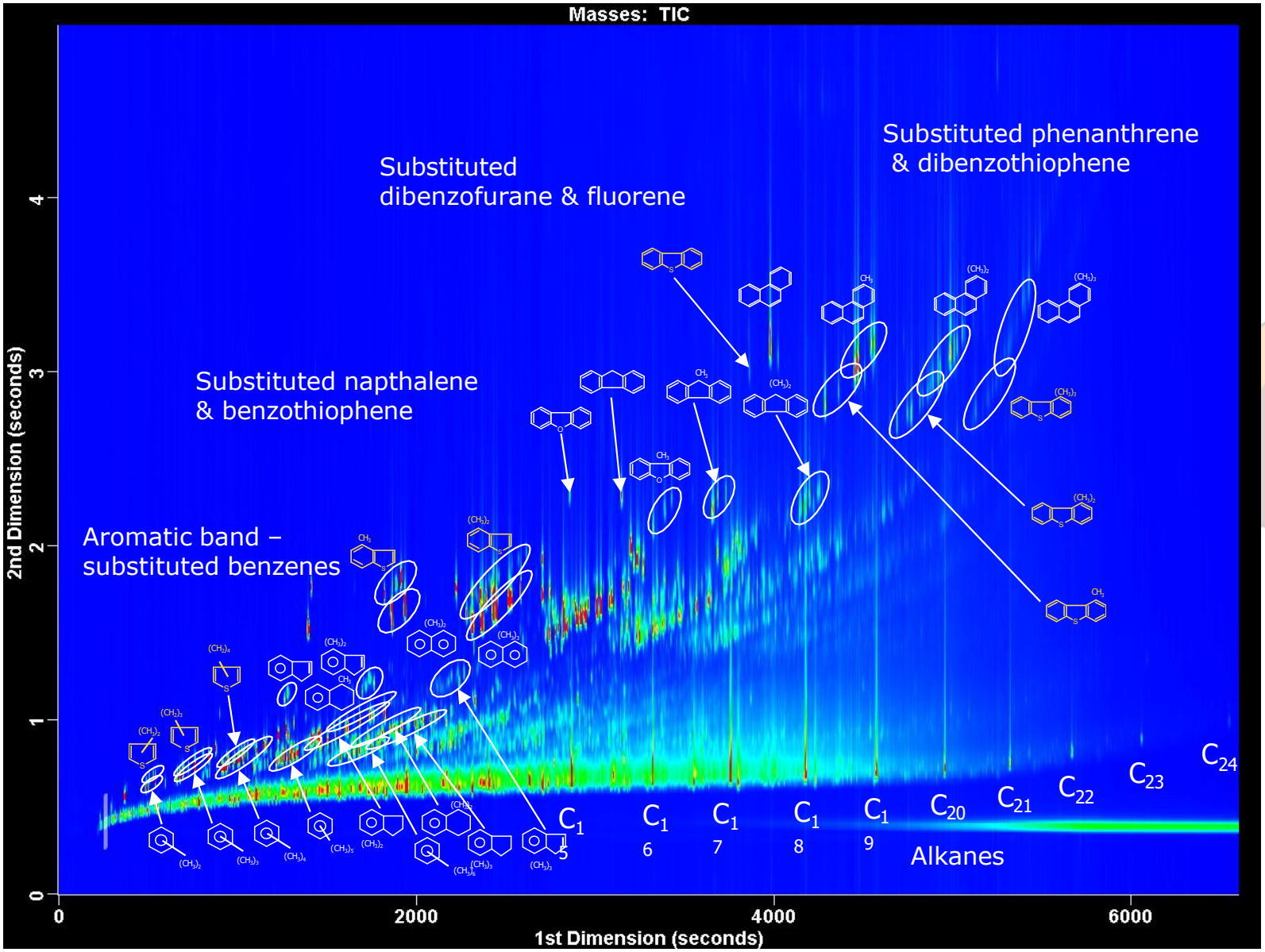
- Gas chromatography is useful for volatile organic compounds with MW<1250 which are:
  - Volatile enough to be vapourised & carried by carrier gas through a GC instrument, usually below 400°C
  - Do not decompose at temperature required to vapourise sample
  - Only around 20% of known organic compounds can be analysed by GC!
- Liquid chromatography is useful for non volatile organic compounds with no real upper mass limit
  - Are soluble in mobile phase
  - Have a lower vapour pressure than sample solvent & mobile phase
  - Detectable!
    - Need chromophore for UV-Vis
    - Need to know absorbance & emission spectra for fluorescence & therefore contain a fluorophore
    - Must be ionisable for LC-MS



- Diesel analysis by direct injection GCxGC-TOFMS

Elemental analysis

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"Elemental analysis is a process where a sample of some material (e.g. soil, waste or drinking water, bodily fluids, minerals, chemical compounds) is analysed for its elemental and sometimes isotopic composition. Elemental analysis can be qualitative (determining which elements are present) and it can be quantitative (determining how much of each are present)."

Types of elemental analysis

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- Atomic Absorption Spectroscopy (AAS), atoms only:
  - Flame Atomic Absorption Spectroscopy (FAAS)
  - Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)
  - Hydride Generation Atomic Absorption Spectroscopy
- Atomic Emission Spectroscopy (AES), atoms only:
  - Flame Photometry
  - Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES), atoms & ions
- Inductively Coupled Plasma – Mass Spectrometry (ICP-MS), ions

Example: ICP-OES of Motor Oil

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During production & analysis post-use

Guideline limits (ppm) for lubricating oil wear metals in different engine components						
	Hydraulic	Gearbox	Diesel Engine	Gasoline Engine	Transmission	Differential
Iron	75	300	80	300	300	1000
Chromium	5	n/a	25	40	10	n/a
Lead	20	n/a	50	n/a	50	n/a
Copper	75	250	50	75	400	250
Tin	10	250	25	40	20	250
Aluminium	25	250	30	40	50	250
Nickel	5	n/a	10	15	20	n/a
Silver	5	n/a	5	5	5	n/a
Silicon	75	250	25	50	50	250

Courtesy of Agilent Technologies

Spectroscopy

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"The study of physical systems by the electromagnetic radiation with which they interact or that they produce. Spectroscopy is the measurement of such radiations as a means of obtaining information about the systems and their components. In certain types of optical spectroscopy, the radiation originates from an external source and is modified by the system, whereas in other types, the radiation originates within the system itself."

In 1672 Isaac Newton used the word spectrum when experimenting with light passing through a prism

In early 1800s Joseph Von Fraunhofer made advances with dispersive spectrometers that improved spectroscopic precision and quantitation

Types of infrared analysis

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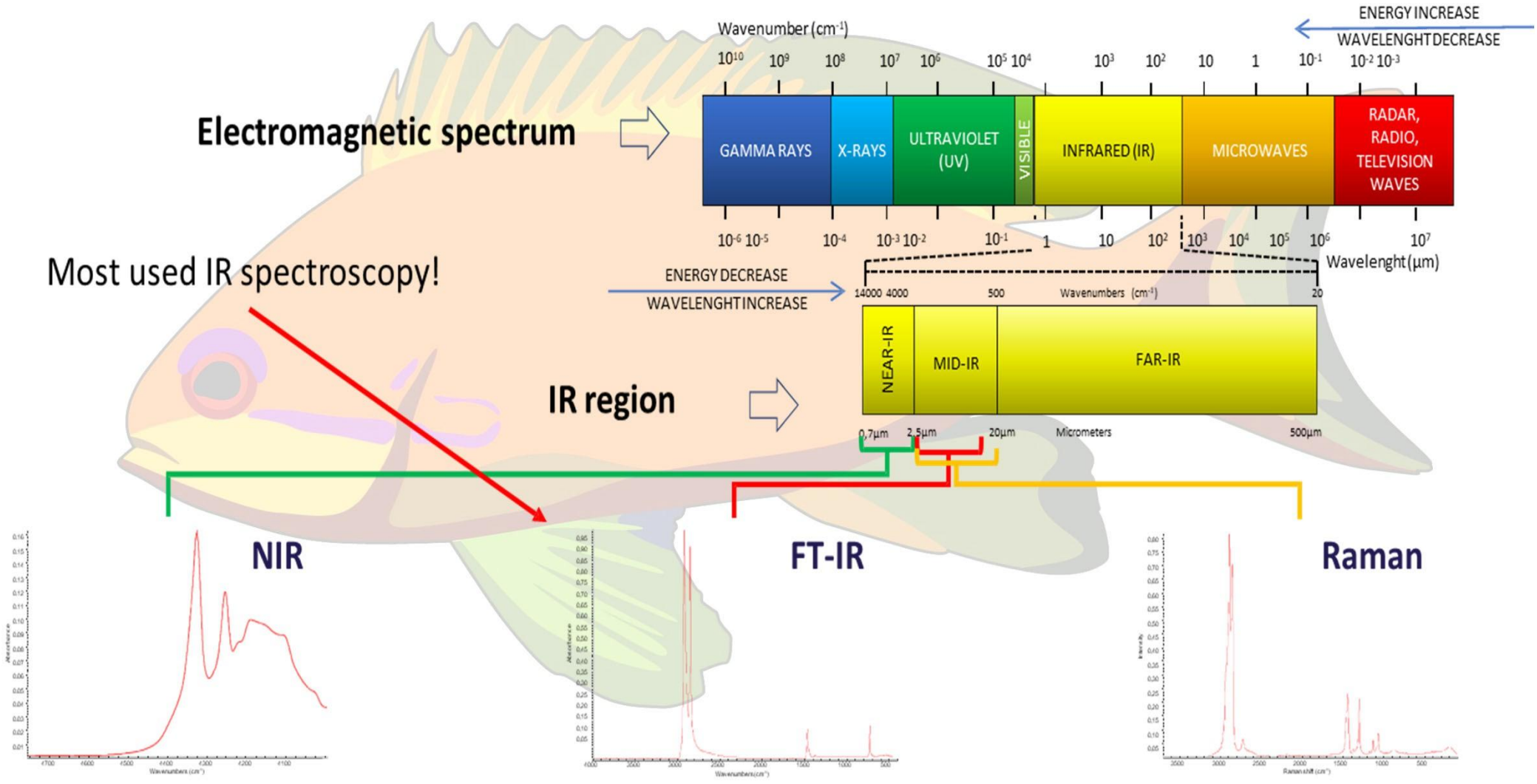

Mass spectrometry

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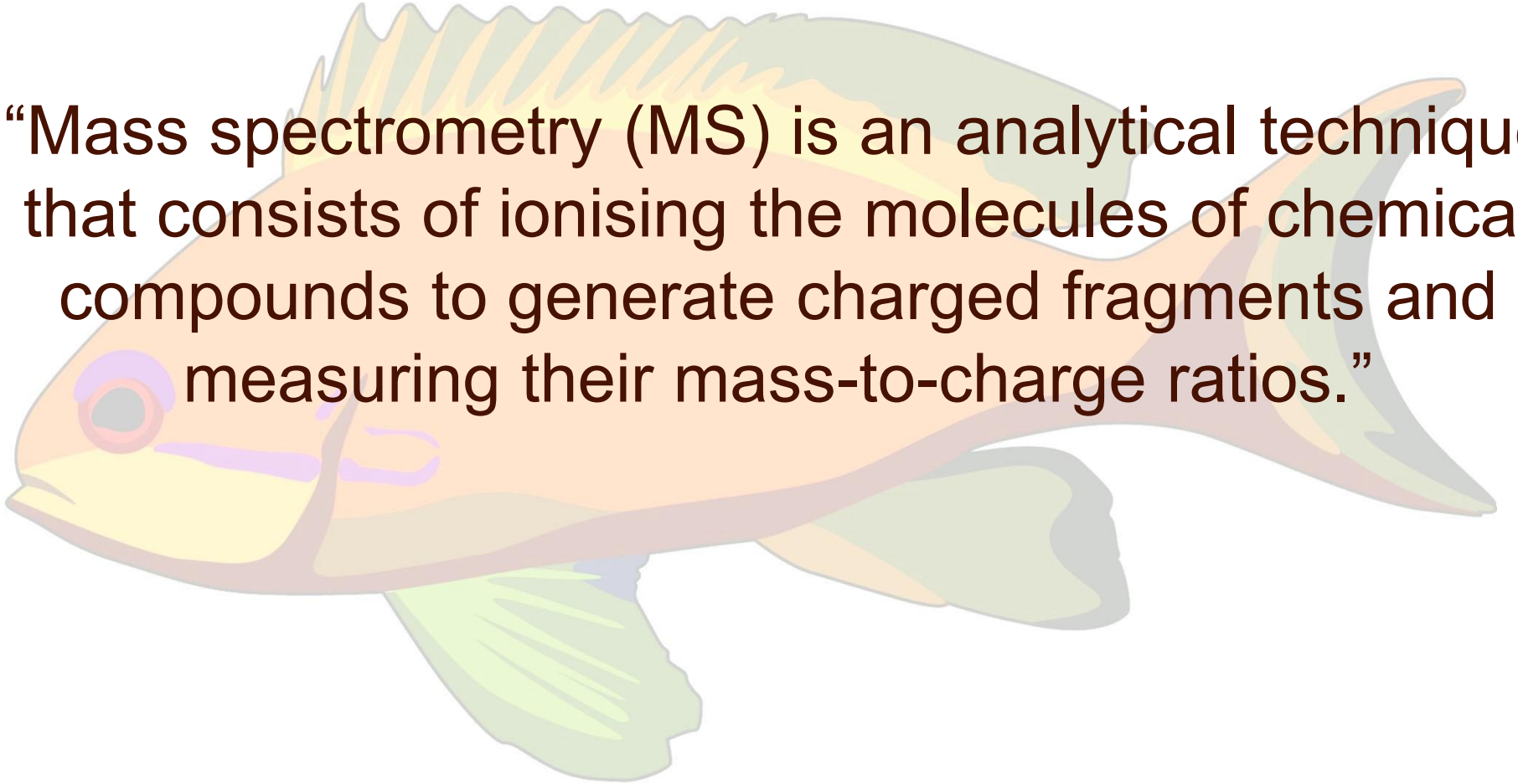

"Mass spectrometry (MS) is an analytical technique that consists of ionising the molecules of chemical compounds to generate charged fragments and measuring their mass-to-charge ratios."

Hyphenation of mass spectrometry

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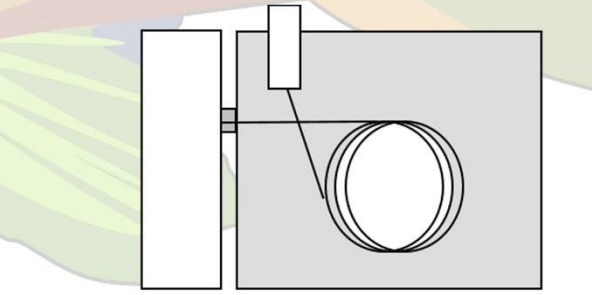
- Mass spectrometry is a technique that can be hyphenated to GC (GC-MS), HPLC/UPLC (LC-MS) & ICP (ICP-MS) used to:
  - Identify unknown analytes
  - Quantify known analytes
  - Determine structural & chemical properties of molecules



GC-MS or LC-MS produces 3D data

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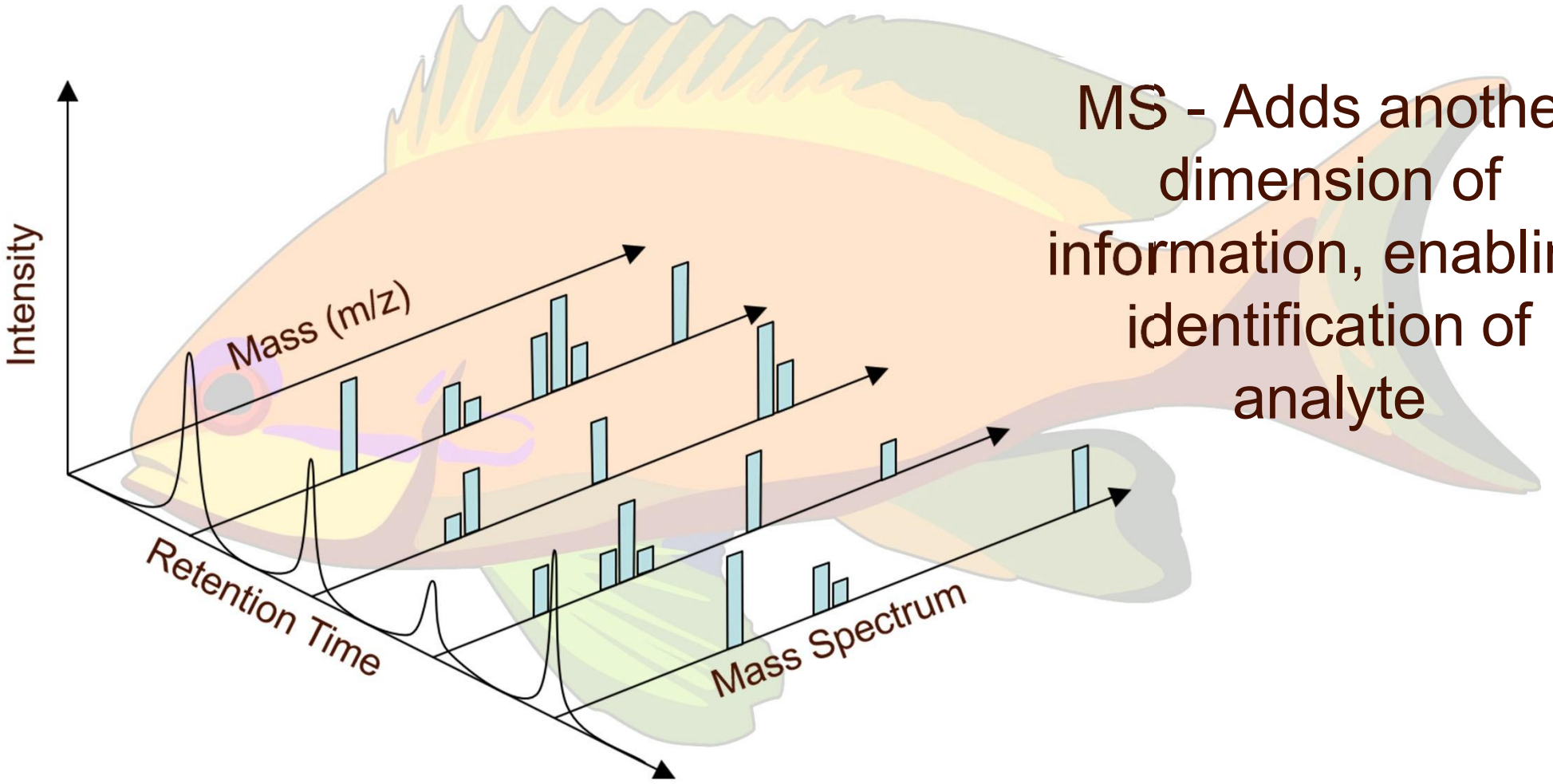
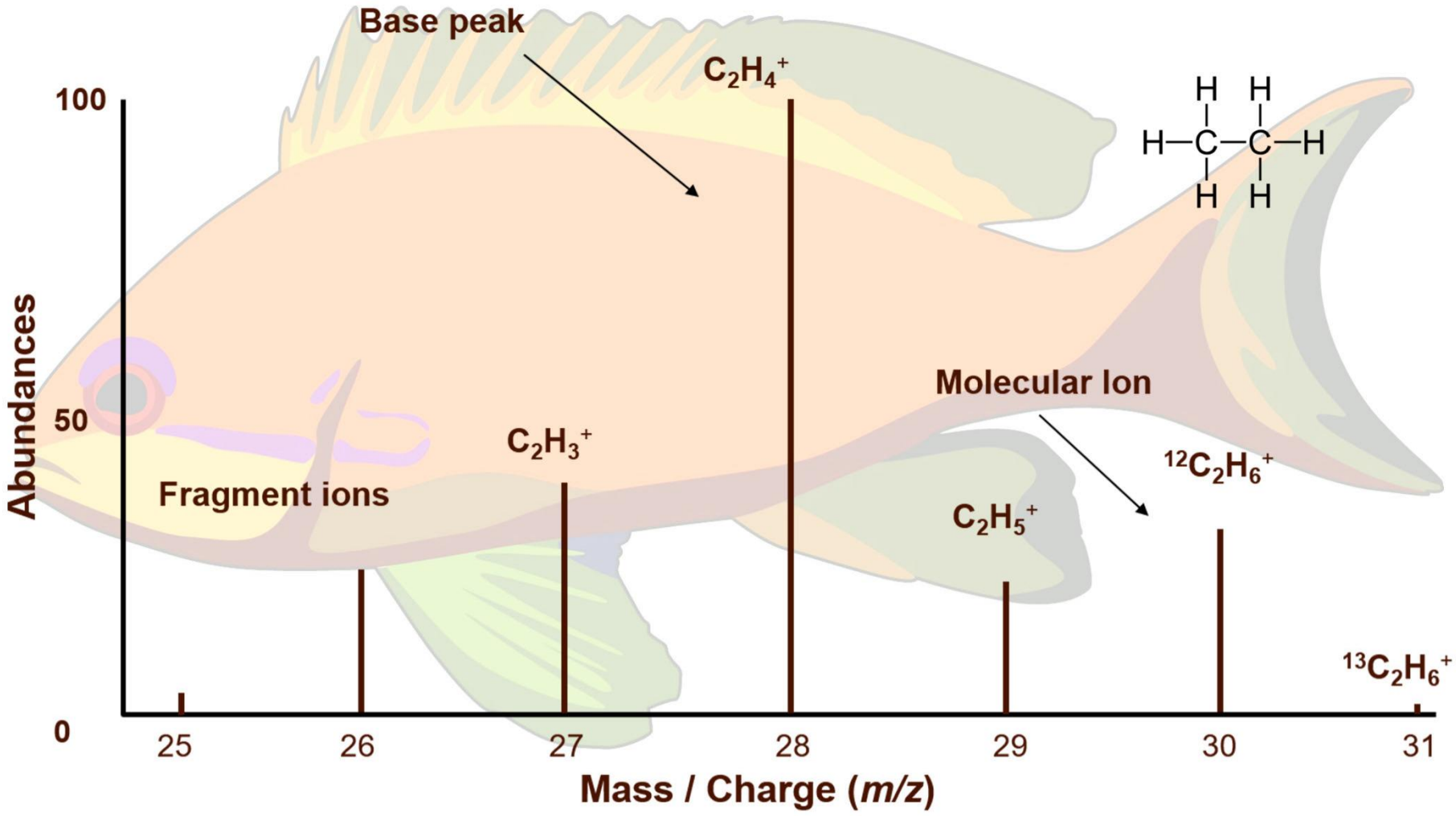


MS - Adds another dimension of information, enabling identification of analyte

GC-MS mass spectrum: Ethane



SPME-GC-TOFMS of tequila

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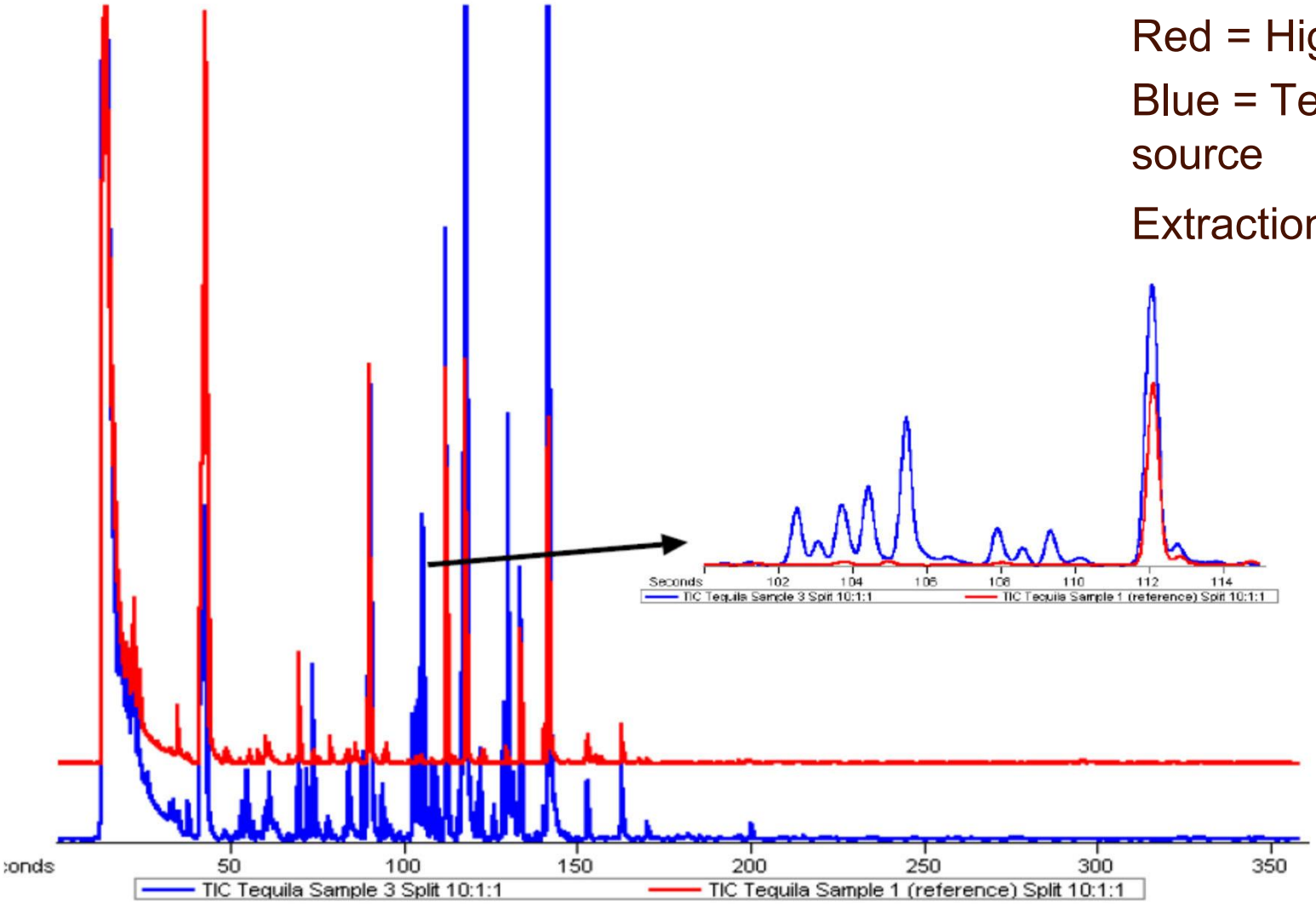
Comparison of two Tequila samples by SPME-GC-TOFMS

Red = High quality tequila

Blue = Tequila from another source

Extraction time = 10 mins



Courtesy of Leco Instruments

ICP-MS mass spectrum

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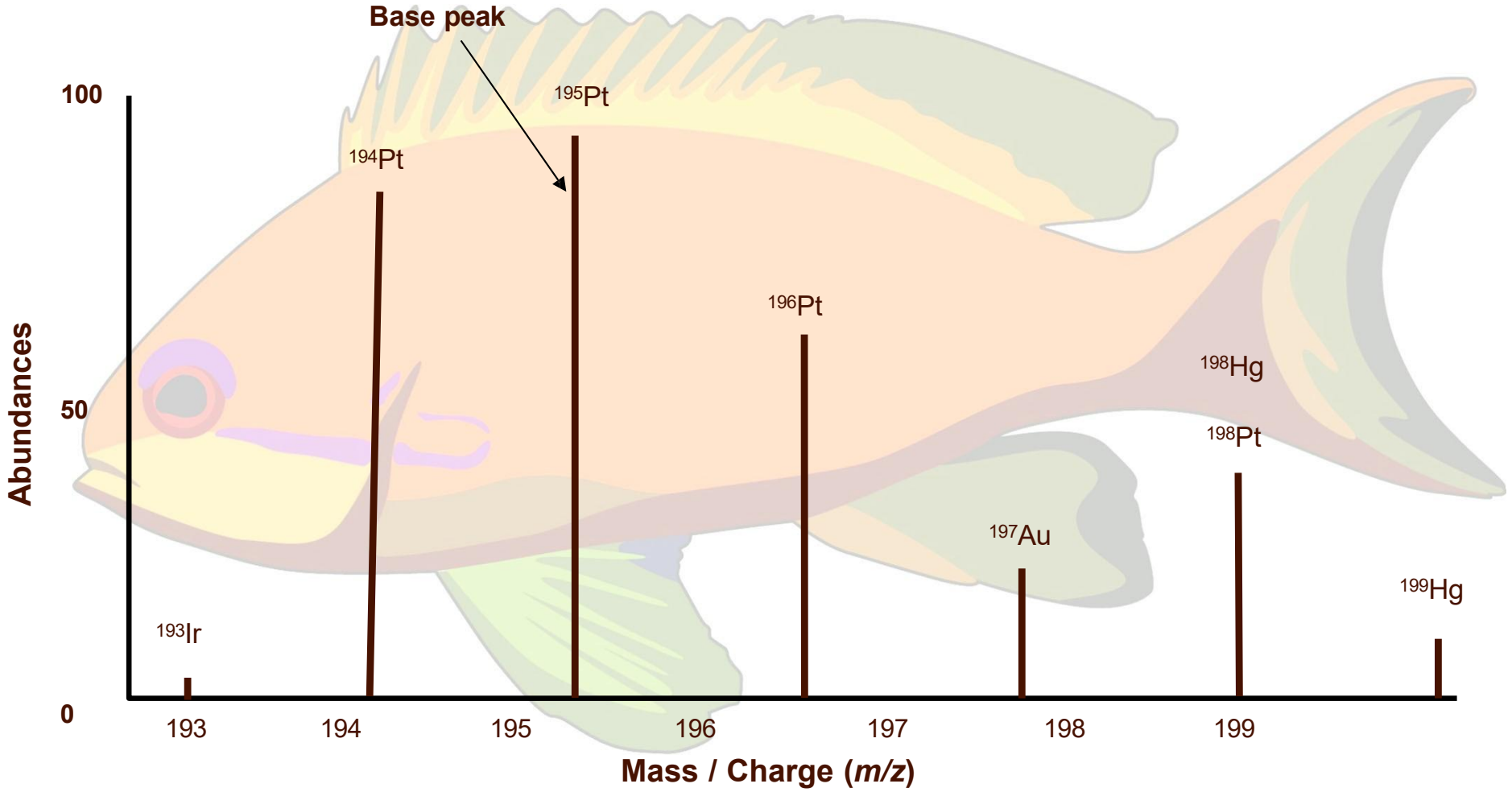

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ICP-MS: Wine authenticity

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Table II: Element concentrations in wine samples

	Red Wines (ppb)		White Wines (ppb)			
Element	Montalcino	Chianti	Magliano	Gavi	Critone	Lugana
^{51}V	3.5	3.5	5.3	2.5	1.4	3.1
^{52}Cr	15.6	14.7	16.5	1.3	4	4.2
^{55}Mn	4160	2808	4660	2208	844	528
^{56}Fe	1176	2660	1660	152	720	289
^{60}Ni	105	76.4	92.8	77.6	15.8	11.3
^{63}Cu	162	281	540	56	7.7	34.8
^{66}Zn	652	800	1068	540	484	440
^{75}As	1.8	1	1.8	1.4	2.6	2
^{78}Se	6.5	2.8	4.8	0.5	0.8	0.5
^{111}Cd	2.8	0.8	0.8	0.3	0.2	0.1
^{118}Sn	12	4.4	1.2	1.8	2.5	1.4
^{133}Cs	12.8	21.4	71.2	4.4	1.5	28
^{205}Tl	0.9	1	1.7	0.2	0.2	0.7
^{208}Pb	14.4	8.9	8.7	3.6	7	16.2

Courtesy of Agilent Technologies

MALDI-TOF mass spectrum: protein

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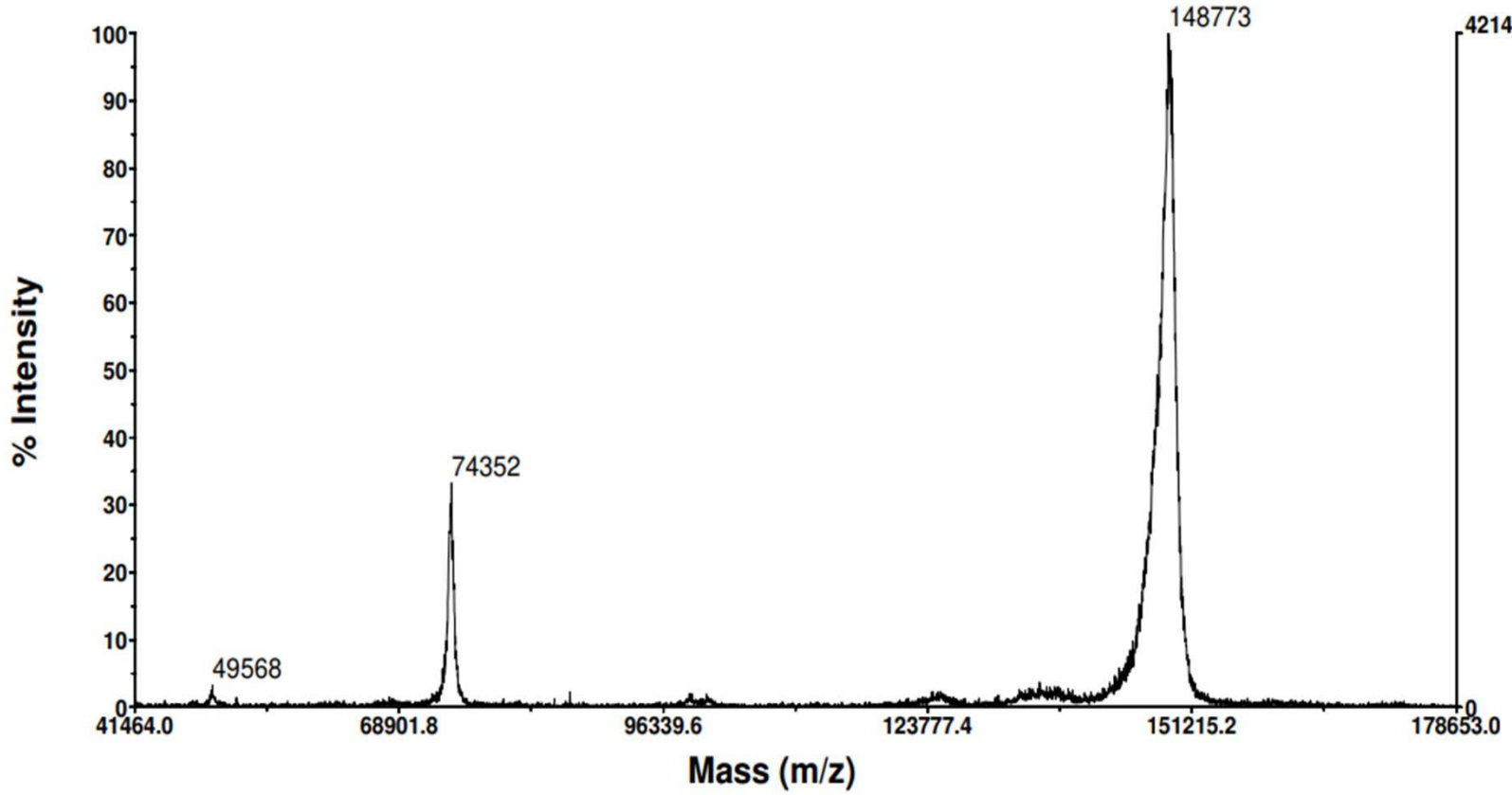
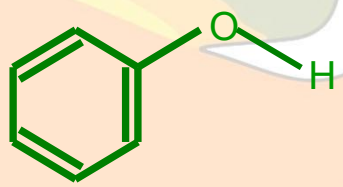
- IgG antibody: mostly singly charged (for such a large molecule!), no fragmentation

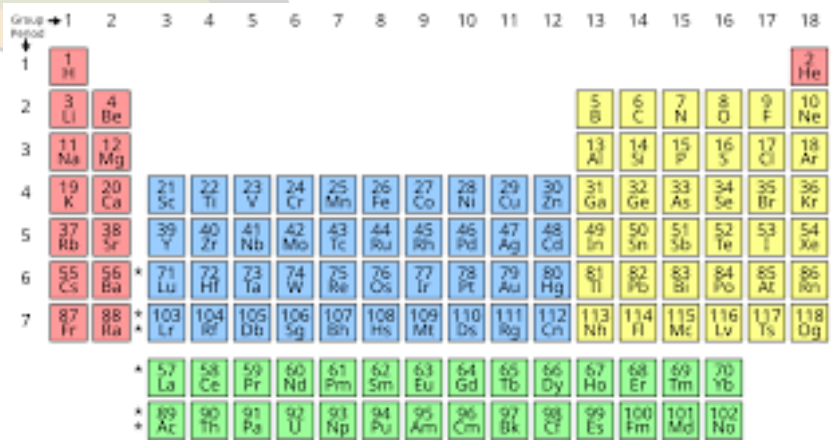


Samples themselves

- Analytical technique chosen depends on
  - Chemical properties & characteristics of compounds to be analysed
    - Elements
    - Volatility
    - Functional groups
  - Questions being asked
- Not so dependent on industry!



$H_3C—OH$



Sampling & sample preparation

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- Samples infrequently directly introduced into analytical instrument
- Most need some type of pre-extraction of analytes from matrix
- Many sampling techniques, which to use depends on:
  - Sample: gas/liquid/solid or somewhere in-between?
  - Analytes: volatile/semi-volatile/involatile
  - Matrix components
  - Where is sample? Can a portion be moved to lab or must be sampled in-situ (can instrument be taken to it)?
  - Number of samples: possible to automate sampling/extraction technique?

Thermal techniques

- Samples: gas, solid, viscous liquid
- Analytes: volatiles to semi-volatile
- **Thermal desorption**: gaseous sample drawn through TD tube filled with packing material – either in lab or other side of world!
- **Thermal extraction**: a small piece of solid or viscous sample is placed in an empty TD tube
- Once in TD instrument:
  - TD tube is heated & analytes concentrated onto a small, cold trap
  - Cold trap is rapidly heating to quickly transfer analytes onto GC column as usual for analysis!
- Thermal desorption & extraction heats sample to a maximum of 350°C & doesn't break any bonds
- Pyrolysis heats solid samples > 350°C breaking C-C bonds

HS and P&T

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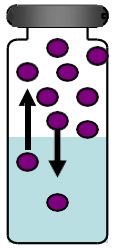
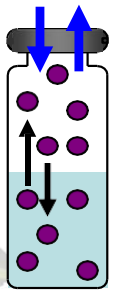
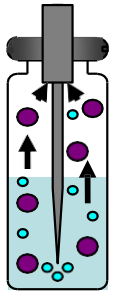
- Samples: liquid (solid/viscous – crush/dilute)
- Analytes: volatile to semi-volatile
- **Static headspace**: sample heated/shaken in sealed vial & portion of gas phase injected



- **Dynamic headspace**: sample swept with gas & analytes trapped on cold trap, thermally desorbed transferring analytes to GC



- **Purge & Trap**: gas bubbled through sample & analytes trapped on cold trap;thermally desorbed transferring analytes to GC



- Sensitivity: P&T > DynamicHS > StaticHS

Food packaging

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Sample: Cookie Wrapper 325 cm²

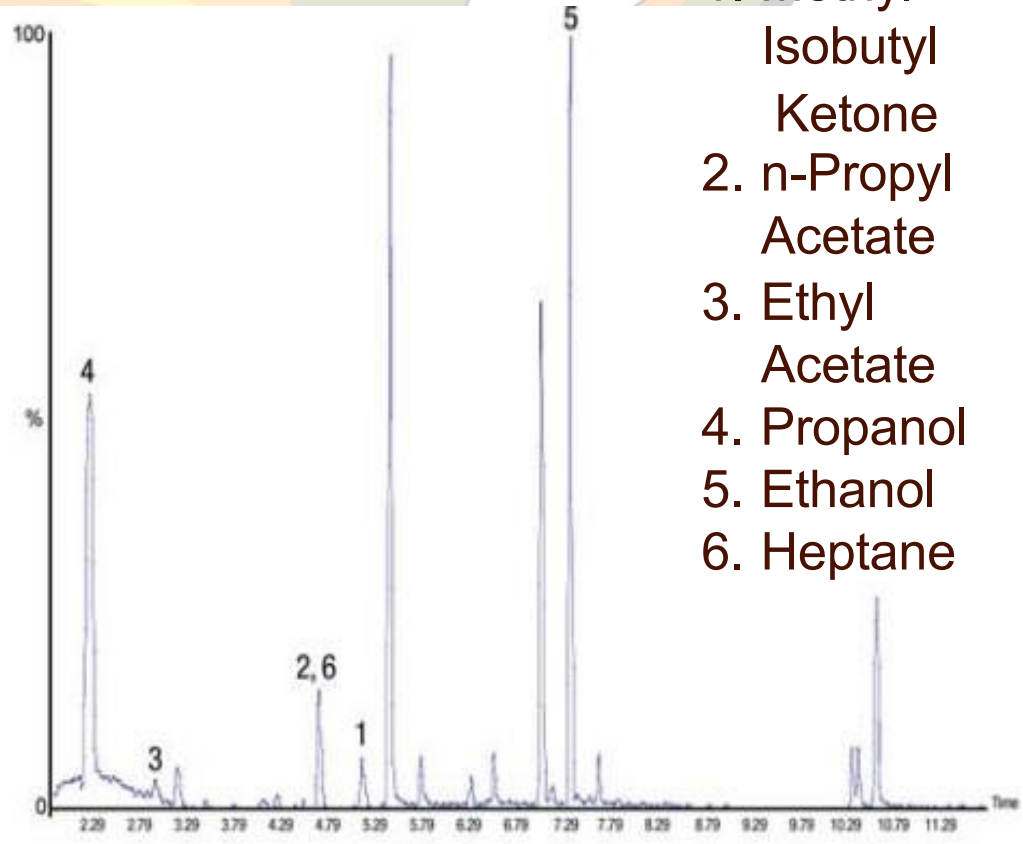
Peak #	Solvent Name	RT (min)	Area	µg in Vial	mg/m²
1	MIBK	5.112	30410	0.01	0.00
2	NPAC	4.664	2320430	0.56	0.02
3	ETAC	2.947	472144	0.16	0.00
4	Propanol	2.243	21689300	6.11	0.19
5	ETOH	1.533	2630198	0.39	0.01
6	Heptane	4.692	211345	0.08	0.00
Total					**0.22**

Volatile organics from a cookie wrapper by HS-GC-MS

Incubation temp: 80°C

Incubation time: 30 min

Automated technique eliminates need for mason jars + operators



1. Methyl Isobutyl Ketone
2. n-Propyl Acetate
3. Ethyl Acetate
4. Propanol
5. Ethanol
6. Heptane

Courtesy of PerkinElmer

SPE, SPME & LLE

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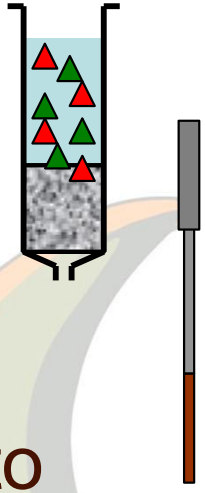

- Samples: liquid (solid/viscous - dissolve/dilute)
- Analytes: volatile to involatile
- Purpose: extract target analytes, remove matrix, concentrate sample, change solvent to GC-amenable
- **Solid phase extraction**: packing material used to trap then eluted using a different solvent
- **Solid-phase micro-extraction**: 1cm fused silica coated with stationary phase (fibre)
  - Different phases used to extract different analytes
  - Placed in sample then desorbed directly in GC inlet/HPLC injector
- **Liquid-liquid extraction**: a non-miscible solvent used

QuEChERS principles

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- Quick, Easy, Cheap, Effective, Rugged & Safe commonly used in food analysis
- Usually prep is:
  - Liquid–solid extraction followed by
  - Liquid-liquid extraction followed by
  - SPE

- Uses d-SPE (dispersive-SPE) e.g. for lipids
- Freeze-out step reduces co-extractives, is faster & easier
- PSA sorbent in d-SPE removes fatty acid co-extractives
- Complex matrix co-extractives reduced using solvents, salts, volumes, adjusting pH & clean-up sorbents
- PTV-LVI GC-MS analysis used to reduce solvents
- Better efficiency & sample throughput with labour reduction, cost savings & reduced waste

Summary

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- Look at the sample from the chemistry side rather than what it is or where it has come from, consider:
  - Chemistry of the components interested in (analytes)
  - Chemistry of the matrix
- Think carefully about what questions are being asked about the sample
- Match the technique(s) to the sample(s) and the question(s)
- Many analytical techniques – far beyond this presentation!
- Understand the technique – RSC approved training
- Fully develop & validate the method
- Obtain accurate & precise results that answer your question!

RSC course approval

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Approval by the Royal Society of Chemistry for continuing professional development (CPD)
https://www.rsc.org/cpd/training

"The objectives of course approval are to highlight good quality training available to the community & encourage members' continuing professional development (CPD)…The approval process is one of peer review, involving assessment against set criteria by members that are experts in their field."

Dr Alice Barker, RSC Accreditation Development Specialist

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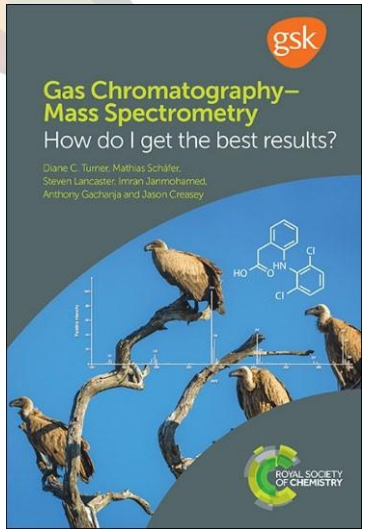
Thank you!

Any questions?

Further reading and information:

Book: http://pubs.rsc.org/bookshop/search?searchtext=Gas+Chromatography-Mass+Spectrometry%3A+How+Do+I+Get+the+Best+Results%3F



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