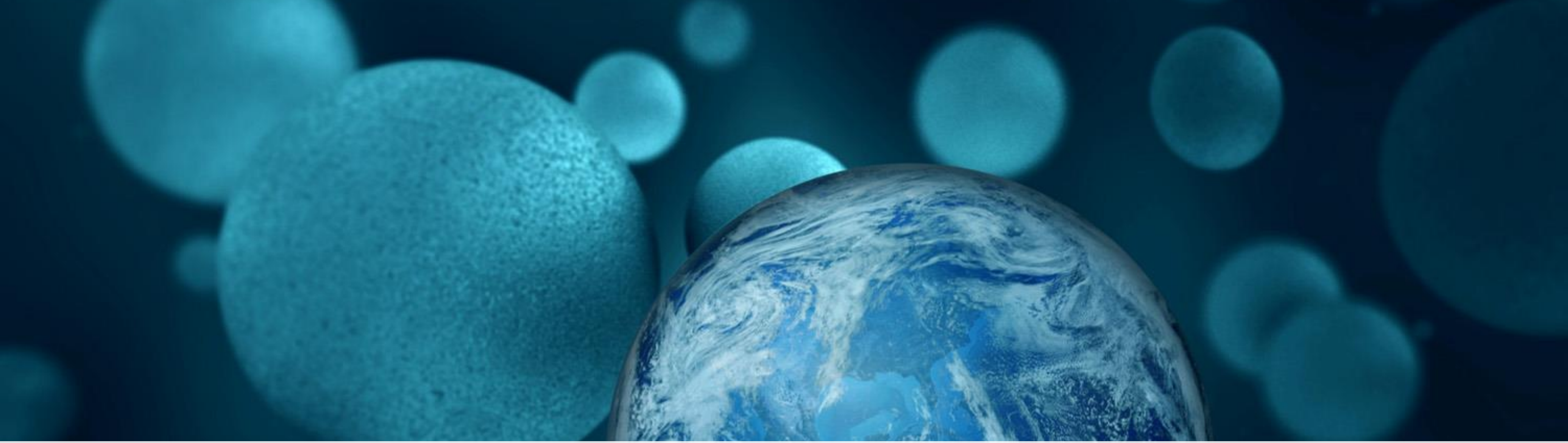

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Food authenticity and adulteration testing using trace elemental and inorganic mass spectrometric techniques

Michal Godula

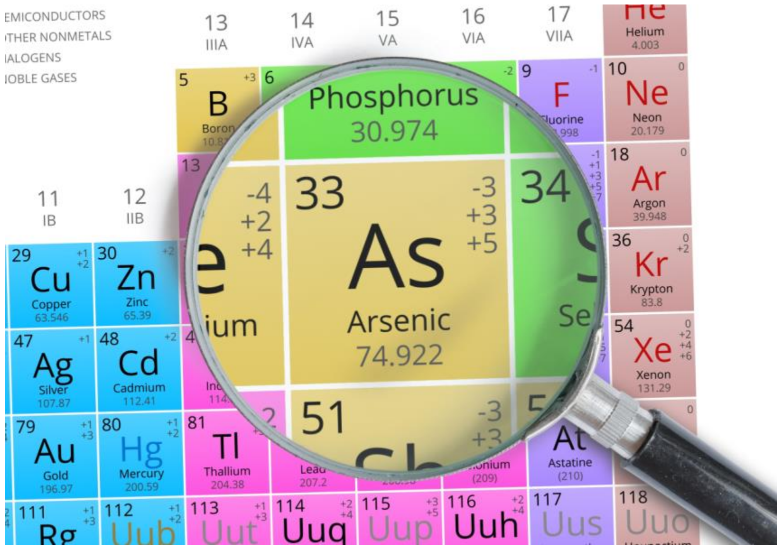
Overview

Food authenticity

- What is food authenticity?
- Major food authentication schemes and systems
- Food authentication challenges
- Which techniques can be applied?
- Example applications

Food safety – focus on arsenic and EU 2015/1006

- What is the EU 2015/1006 regulation and how does it impact arsenic measurement in foodstuffs?
- Why has this legislation been developed?
- What levels need to be measured and in what samples?
- Using IC for speciation analysis and automating speciation analysis
- Application examples: Arsenic speciation in organic brown rice syrup and total arsenic quantification in food samples
- Resources available for learning more about As determination



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What is food authenticity?

- Food authenticity is about misrepresentation by either mislabeling or by adulterating, usually with lower cost material

- Typical examples:
  - Wine authenticity – illegal sugar addition to grape juice
  - Addition of sugar to honey
  - Addition of hazelnut oil to olive oils
  - Mislabeling geographical origin (wine, olive oil)
  - Mislabeling organic food products
  - Adulteration of meat with cheaper species

Major food authentication schemes and systems

- Protected Designation of Origin (PDO)
  covers agricultural products and foodstuffs which are produced, processed and prepared in a given geographical area using recognized know-how.

Council Regulation (EC) No 510/2006 of 20 March 2006

- Protected Geographical Indication (PGI)
  covers agricultural products and foodstuffs closely linked to the geographical area. At least one of the stages of production, processing or preparation takes place in the area.

- Traditional Speciality Guaranteed (TSG)
  highlights traditional character

- DOOR (Database of Origin and Registration) Database
  > 1200 entries for different foods (e.g olive oils, wines, cheeses, honey, beer) from multiple countries

Council Regulation (EC) No 509/2006 of 20 March 2006

See http://ec.europa.eu/agriculture/quality/ and http://ec.europa.eu/idabc/en/document/5360/5637.html for further information

Food authentication challenges

- Chemically identical foods or identical chemical entities
- Unique marker compounds rarely found - more often small analytical differences (isotopic patterns)
- Large natural variability based on climatic conditions, fertilizers used, variety, processing……
- Techniques must be able to distinguish small differences
- Databases of authentic foods must be available to understand natural variability

Which techniques can be applied?

Technique	Parameters measured
Infrared spectroscopy (NIR, MIR)	**Spectral profile, characteristic frequencies**
Raman spectroscopy	**Spectral profile, characteristic frequencies**
Nuclear Magnetic Resonance	**Spectral profile, characteristic frequencies**
Atomic absorption and emmision spectroscopy, ICP/MS	**Elemental composition**
Mass spectrometry (GC/MS, LC/MS)	**Volatile components, phenolic compounds**
Chromatography (HPLC/RID)	**Amino acids, carbohydrates, phenolic compounds**
Isotope Ratio Mass Spectrometry (IRMS)	**Isotope Ratios of natural variations**

DNA based methods like PCR; used mainly for adulteration of meat based products e.g. addition of chicken and pork meat into minced beef, differentiation of cattle breed, adulteration of foie gras with chicken*

Immunology based approach based on ELISA detection of specific proteins e.g. presence of vegetable proteins in milk or meat, adulteration of sheep/goat milk with cow milk

** L.M. Reid et al. / Trends in Food Science & Technology 17 (2006) 344–353*

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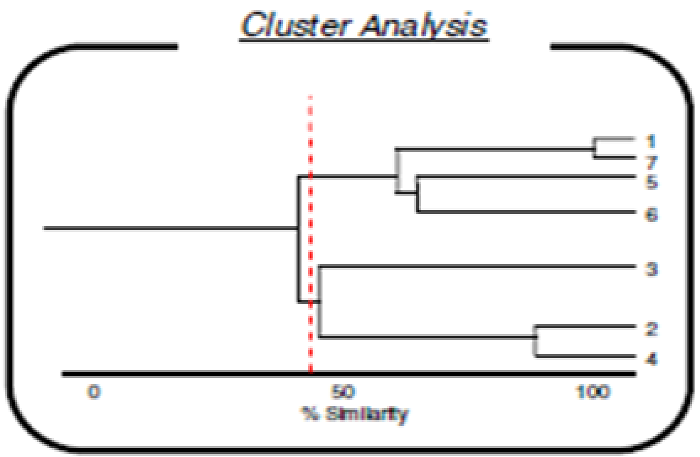
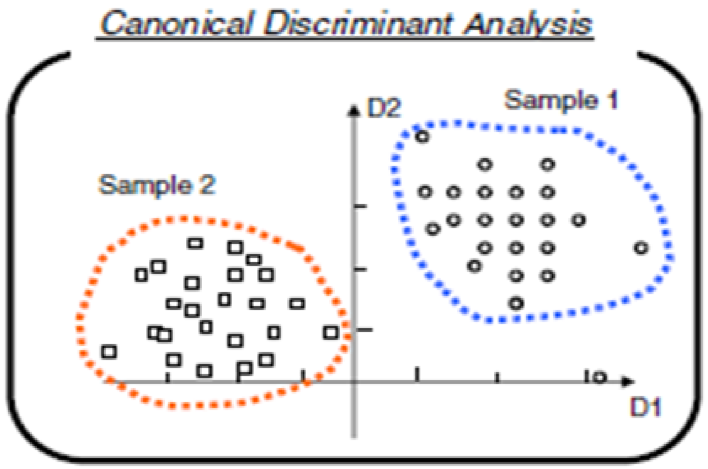
Data analysis: Chemometric approach

- Large number of variables involved, e.g. delta value, elemental composition, mass spectra, compound concentration etc.
- Multivariate analysis must be applied
- Supervised or unsupervised methods
  - Unsupervised: classification of sample without any knowledge about the origin
  - Supervised: similarity of unknown to authentic material
- Choosing appropriate approach is the key to successful analysis



Unsupervised methods

- ANOVA, MANOVA
- Principal Component Analysis (PCA) - reduces dimensionality
- Cluster Analysis (CA) - grouping of samples based on their similarity

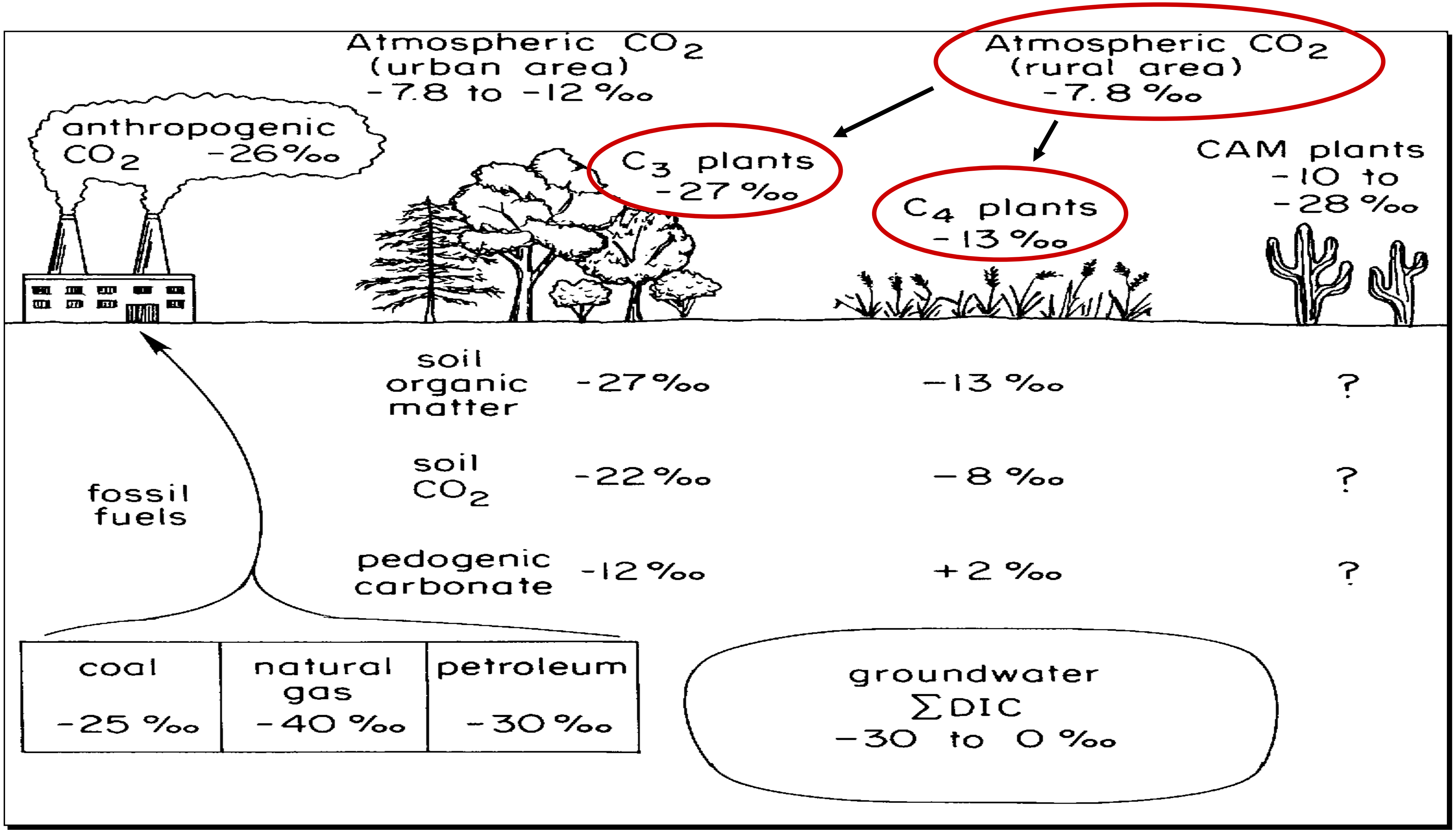


Supervised methods (discriminant techniques)

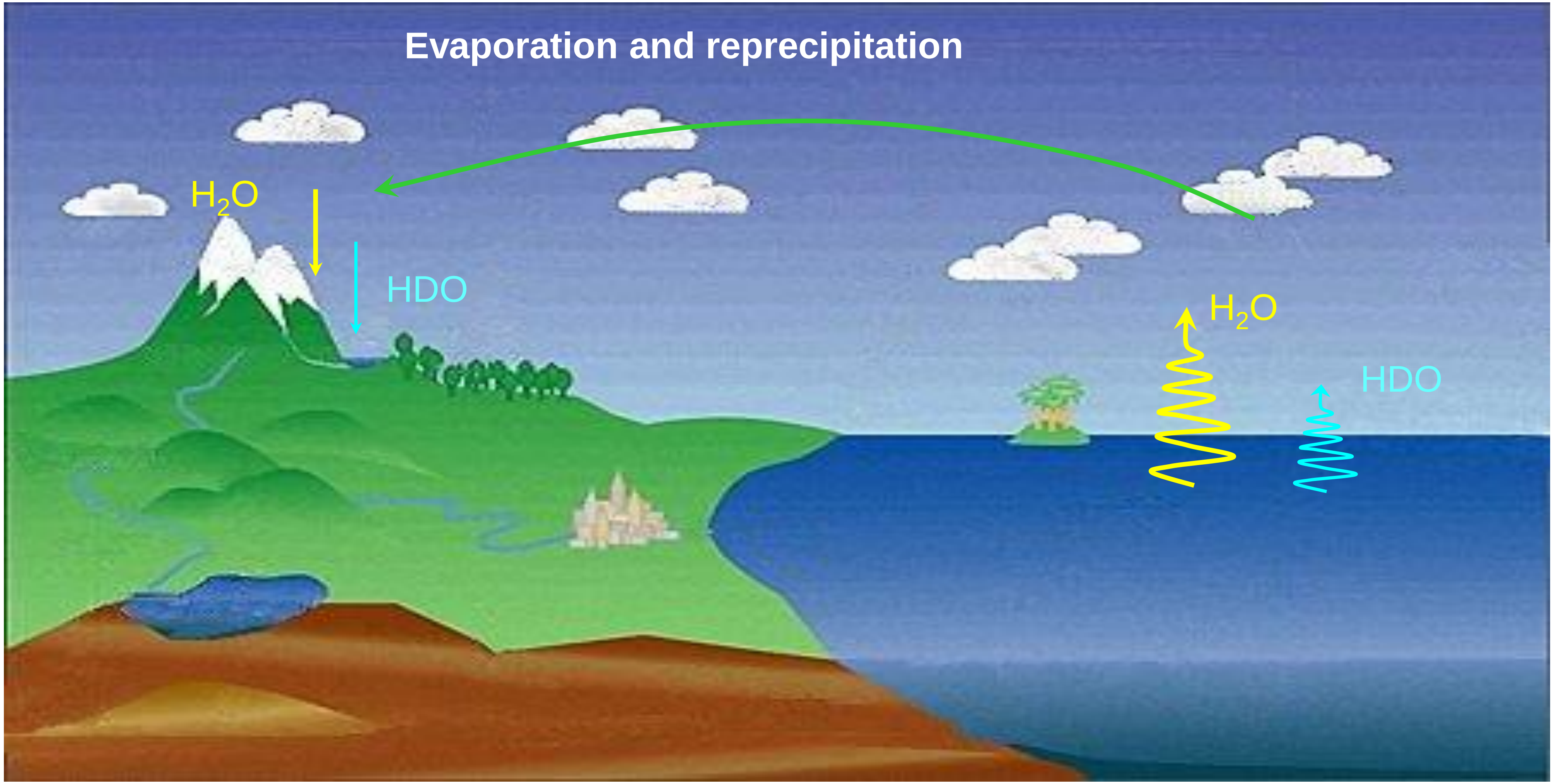
- Linear discriminant analysis (LDA) - characterize or separate two or more classes
- Partial Least Squares (PLS) - fundamental relations between two matrices
- Artificial Neural Networks (ANN) - complex relationships modelling

Gonzalves, A, Trends in Analytical Chemistry, Vol. 28, No. 11, 2009

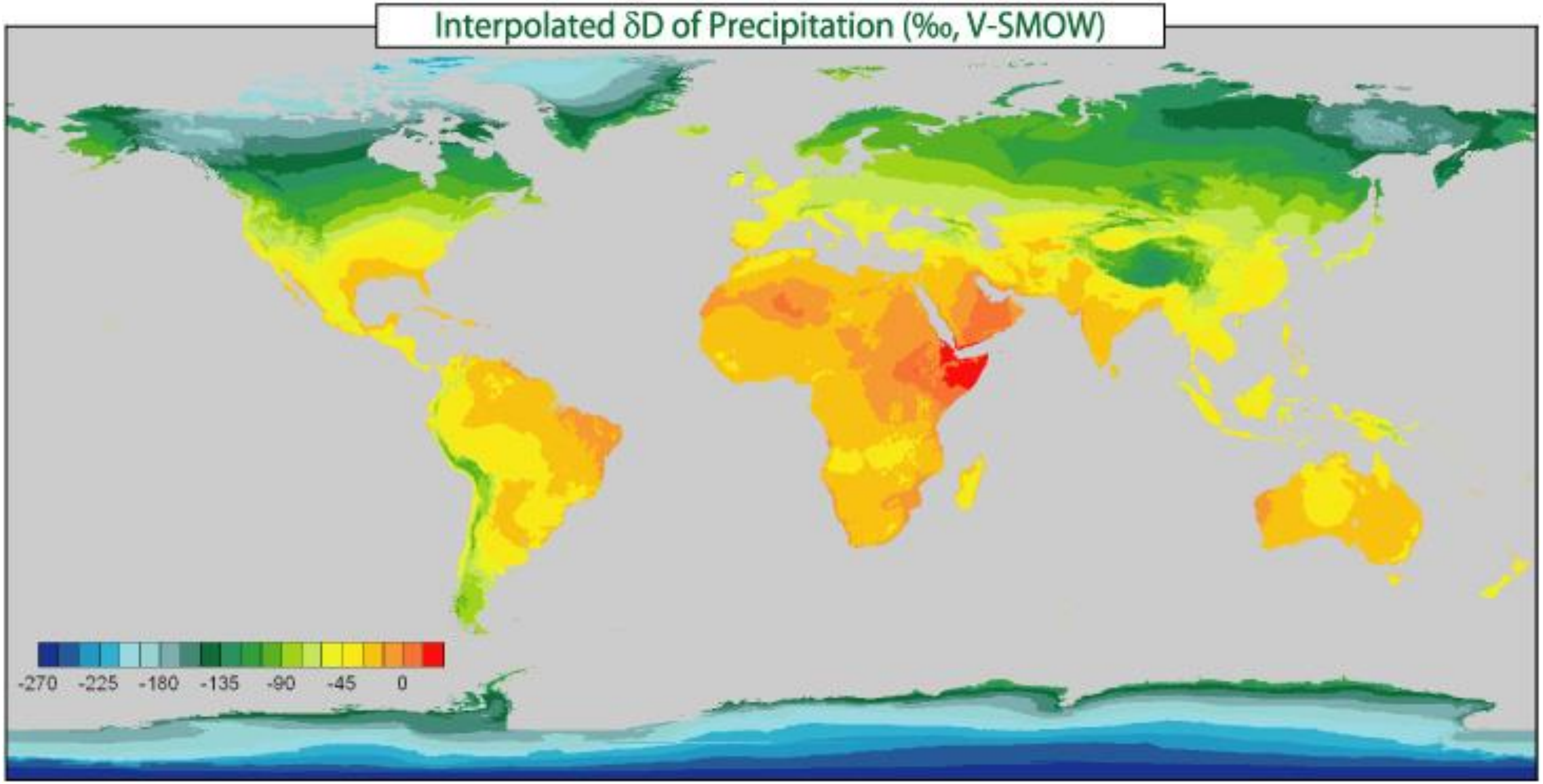
Isotopes - sources of variation



Climatic factors affecting D/H ratio



Interpolated δ^2H of precipitation



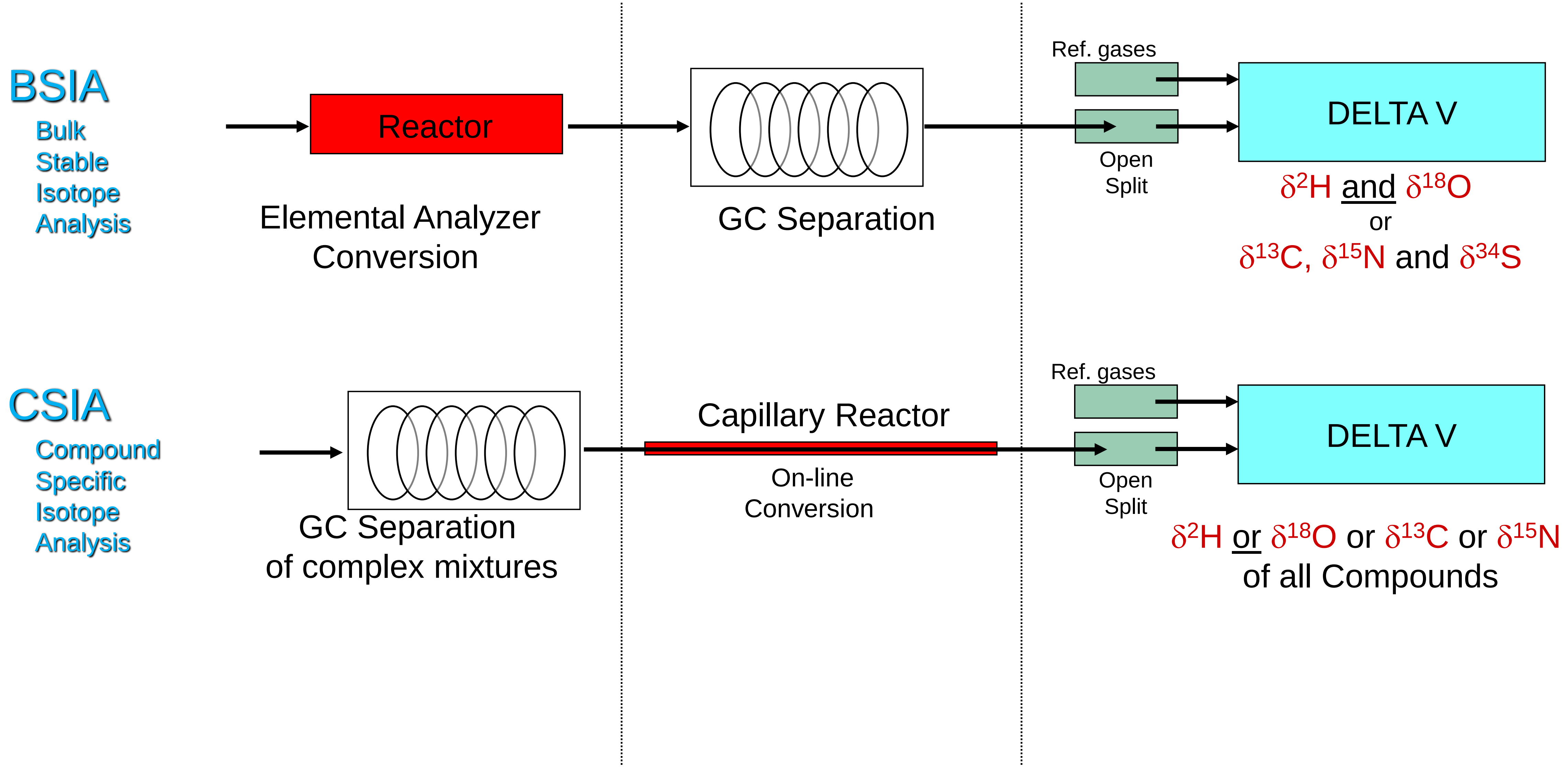
Produced by James Elhringer (University of Utah)

- **Precise isotope ratios of**

Element	Minor Isotope	Natural Abundance [%]
Hydrogen	^{2}H (D)	0.015**57**
Carbon	^{13}C	1,111**40**
Nitrogen	^{15}N	0.366**30**
Oxygen	^{18}O	0.200**04**
Sulfur	^{34}S	4.215**00**

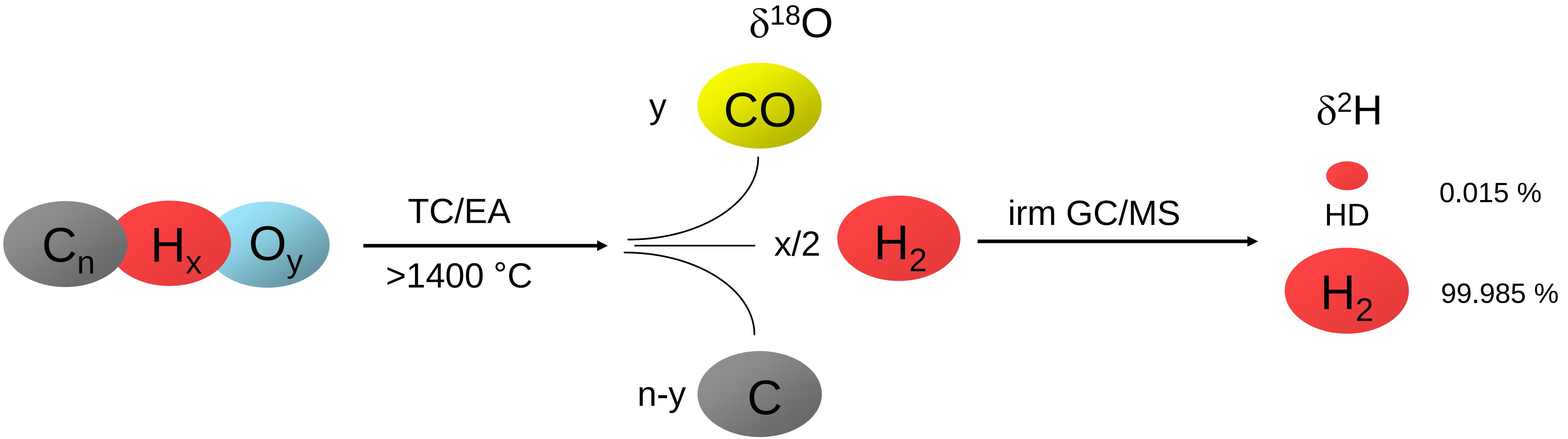
That's where the information is

Different approaches to IRMS

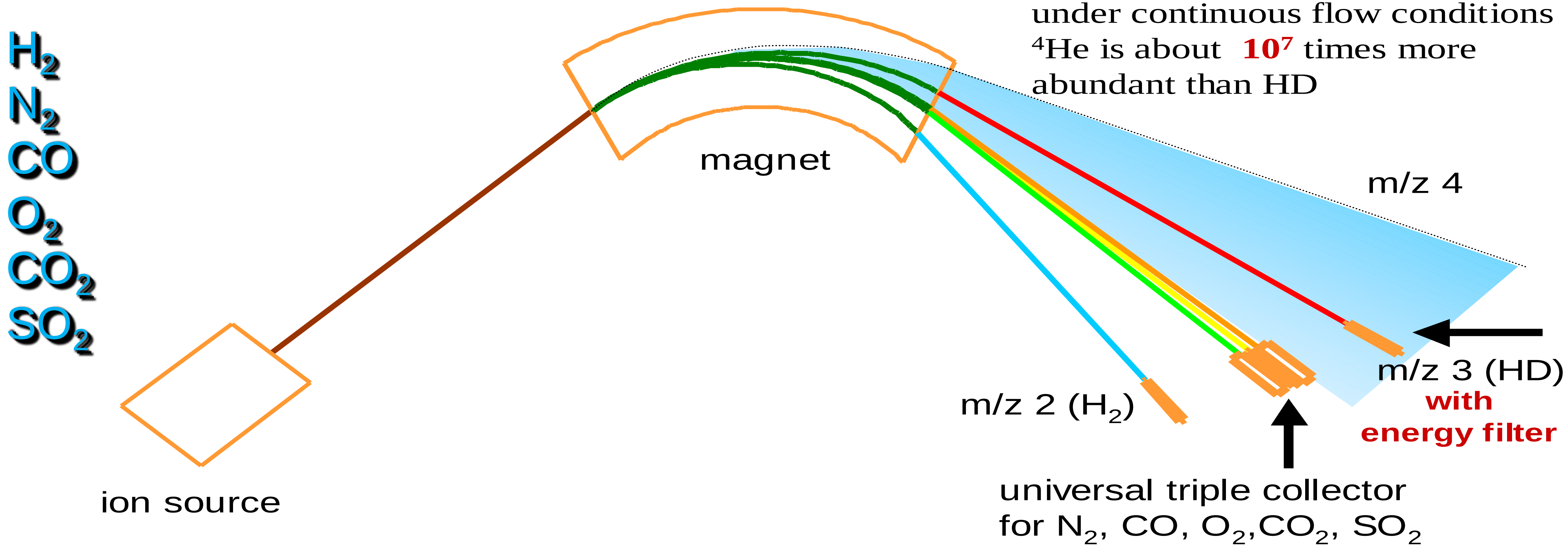


Sample introduction – conversion to simple gases

- BSIA: Dynamic Flash Combustion (EA) for C, N & S → CO_2, SO_2, N_2
- BSIA: High temperature conversion (TC/EA) for O & H → CO, H_2
- CSIA: Combustion (GC/C) for C or N → CO_2, N_2
- CSIA:High temperature conversion (GC/TC) for O or H → CO, H2

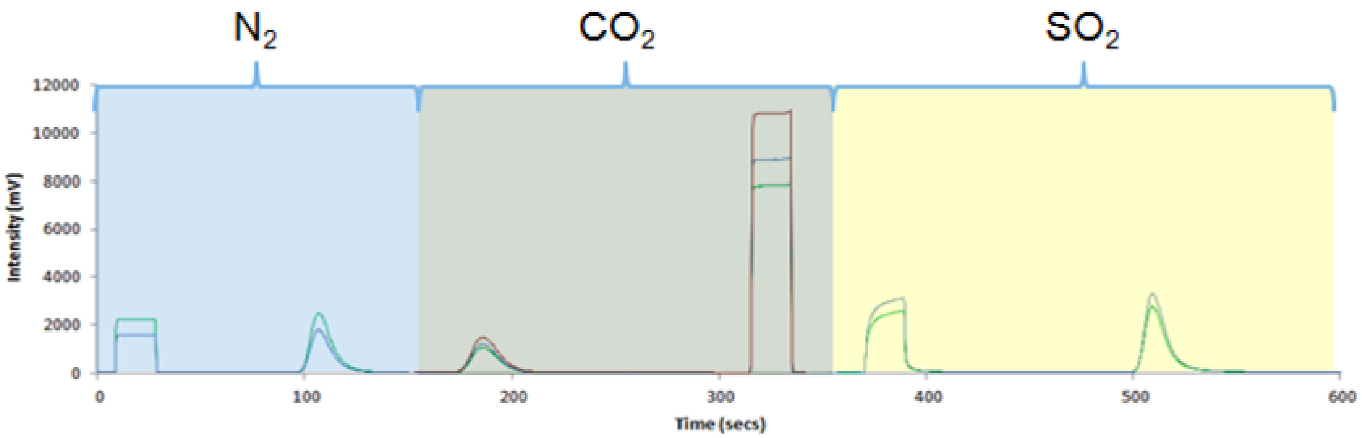


Multi collectors for simultaneous detection



Bulk Stable Isotope Analysis: The Thermo Scientific™ EA IsoLink™ IRMS System

- Allows simultaneous determination of C, N and S or O and H
- CNS analysis in less than 10 mins, including a very small sample amounts (< 5 µg)
- Provides chromatographic profile of the sample AND isotopic ratio information
- Helium Management (HeM) Module offers > 60% He saving
- All-in-one fully automated, modular EA–IRMS solution for all CNSOH applications



Analysis time: **10 mins**
He flow: 100 ml/min
Total He: 1.5 liters per sample

>60% He saving per NCS sample

For further information go to www.thermofisher.com and search for EA IsoLink IRMS

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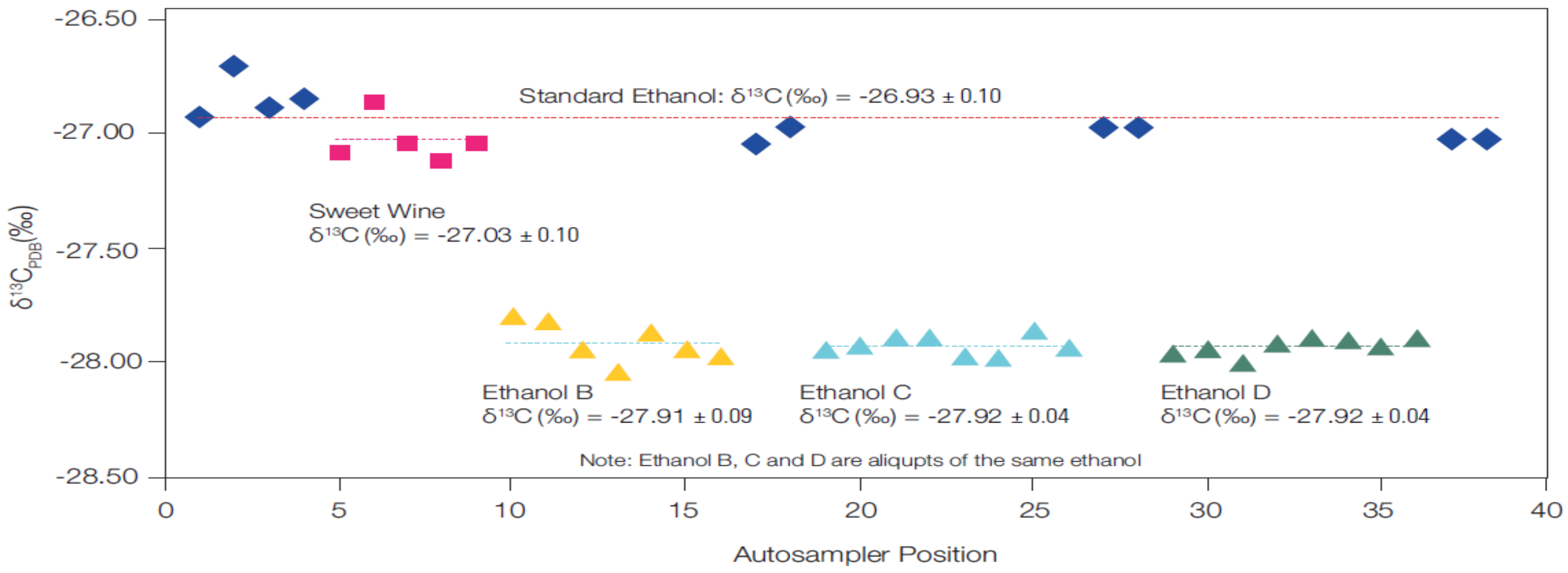
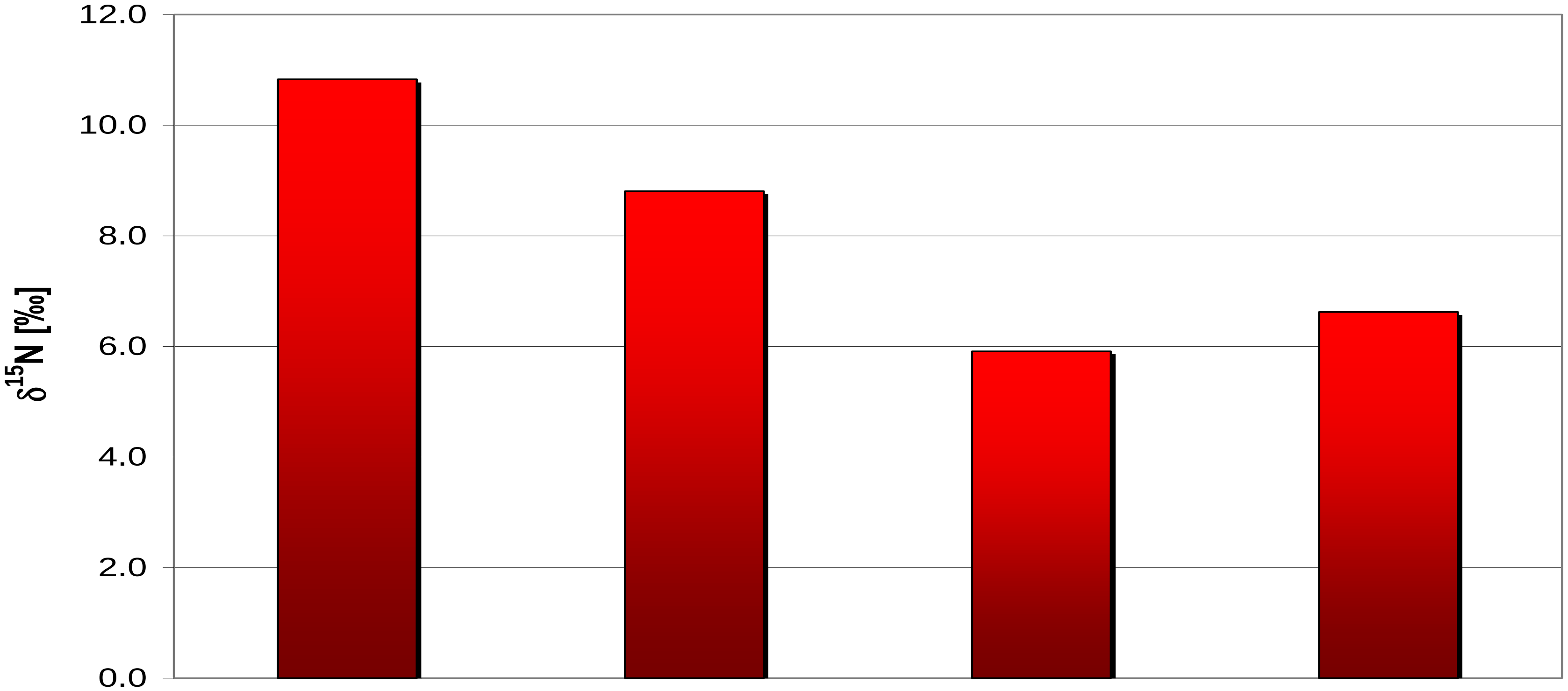
EA–IRMS: C analysis of ethanol



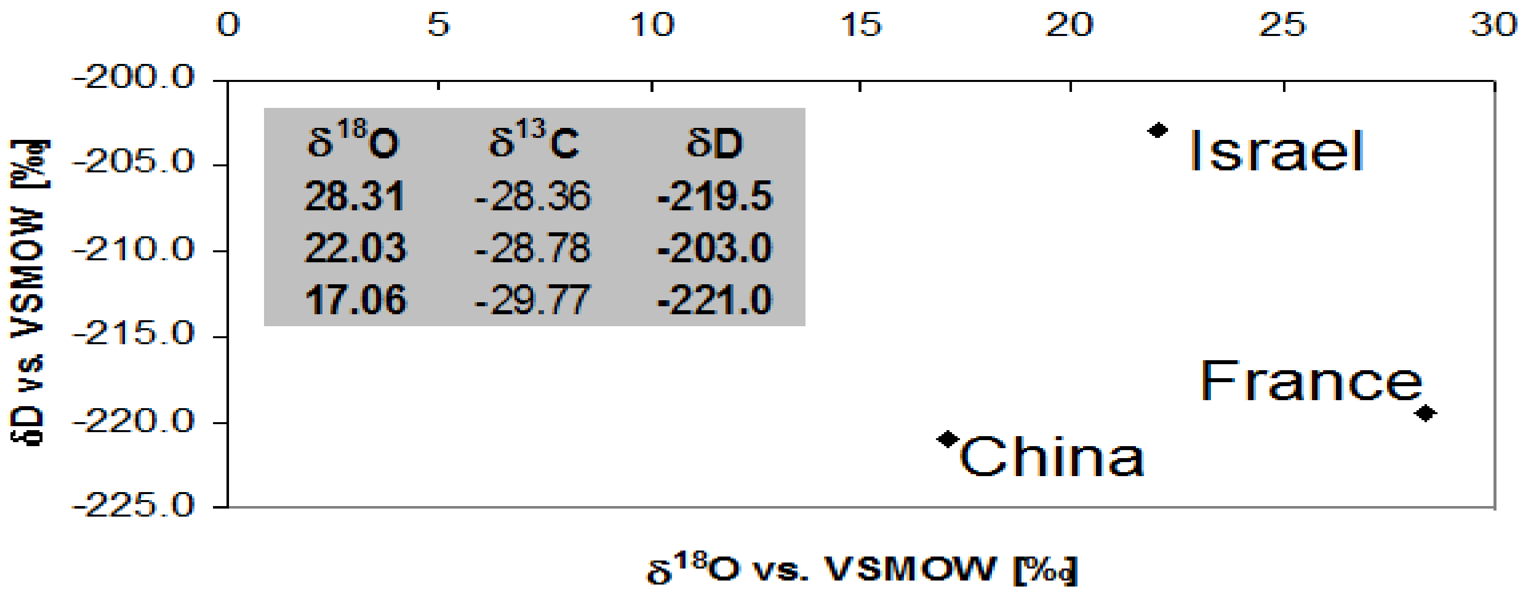
- $\delta^{13}C$ can be used to determine if wines have been adulterated with sugar
  - See Applications note AN30147

EA–IRMS: $\delta^{15}N$ in tomatoes



- Depending on the fertilizer the N isotope signature in plants can differ. Mineral fertilizers show low $\delta^{15}N$ values while organic fertilization by compost results in higher $\delta^{15}N$ values.
- This allows discrimination of organic from non-organic produced vegetables.

EA–IRMS: H and O in hazelnut oil



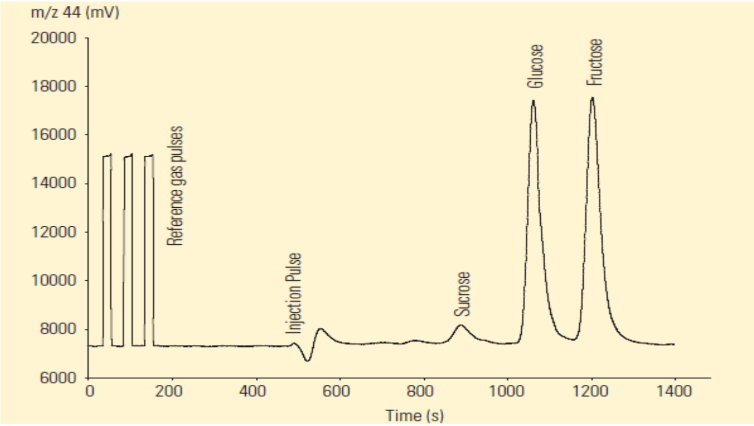
- Edible oils are commonly protected by regional designations (PDO) and subject to strong quality controls. This makes them a target for fraud. The example shows that O and H isotopes can distinguish between oils of different geographical origin.

Extending the application of EA-IRMS: Honey adulteration

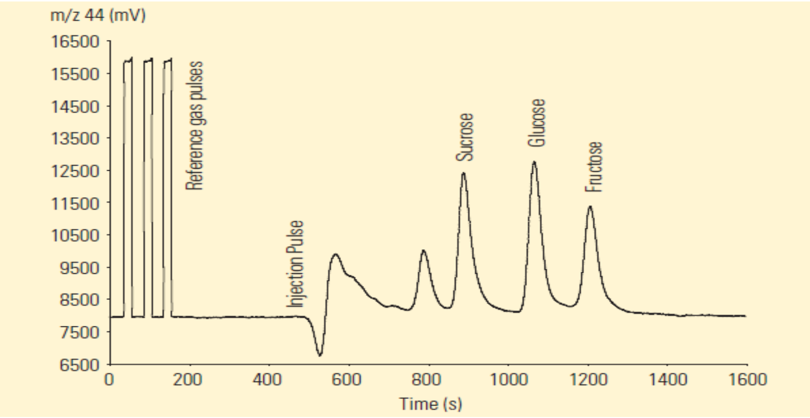
- Problem: Addition of cheap sugars to honey
- Current analytical approach:
  - EA-IRMS to assess $\delta^{13}C$ value of honey and its protein fraction (limit of detection ~ 7% of C4 sugar addition)
  - Low sensitivity for sugars analysis, so need an enhanced approach
- Solution: irm-LC/MS using the LC IsoLink™ interface allows:
  - Comparison of $\delta^{13}C$ value of different sugars
  - Determination of the sugar pattern
  - Higher sensitivity - 100x more sensitive than direct EA-IRMS
- See our application note AN30024 - Testing honey adulteration by $\delta^{13}C$-EA/LC-IRMS for full details

Honey adulteration examples

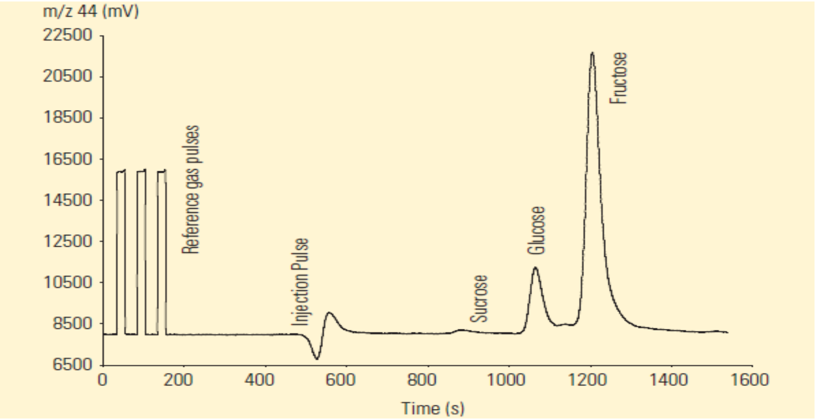
HONEY	SUCROSE ‰	GLUCOSE ‰	FRUCTOSE ‰	FRU/GLU RATIO OF AREAS	EA HONEY(4) ‰	EA PROT.(4) ‰	ADULT.(4) %	
1	-23.3	**-23.2**	**-22.9**	1.07	**-21.8**	-24.2	**16.7**	**adulterated**
2	-11.3	**-11.2**	**-13.9**	**0.65**	**-11.9**	n.a.	n.a.	**adulterated**
3	-25.3	-24.9	-24.9	1.42	-24.8	-24.8	0.0	
4	-26.4	-26.5	-26.4	0.97	-25.4	-21.6	0.0	
5	n.d.	-26.1	-26.0	**4.53**	-25.8	-26.1	1.9	**adulterated**
6	-26.1	-25.0	-25.3	1.62	-24.3	-24.3	0.0	
7	-25.0	-25.2	-25.1	1.16	-24.2	-24.7	3.4	
8	n.d.	**-25.1**	**-26.4**	2.17	-24.8	-25.1	1.5	**adulterated**



Honey 1



Honey 2

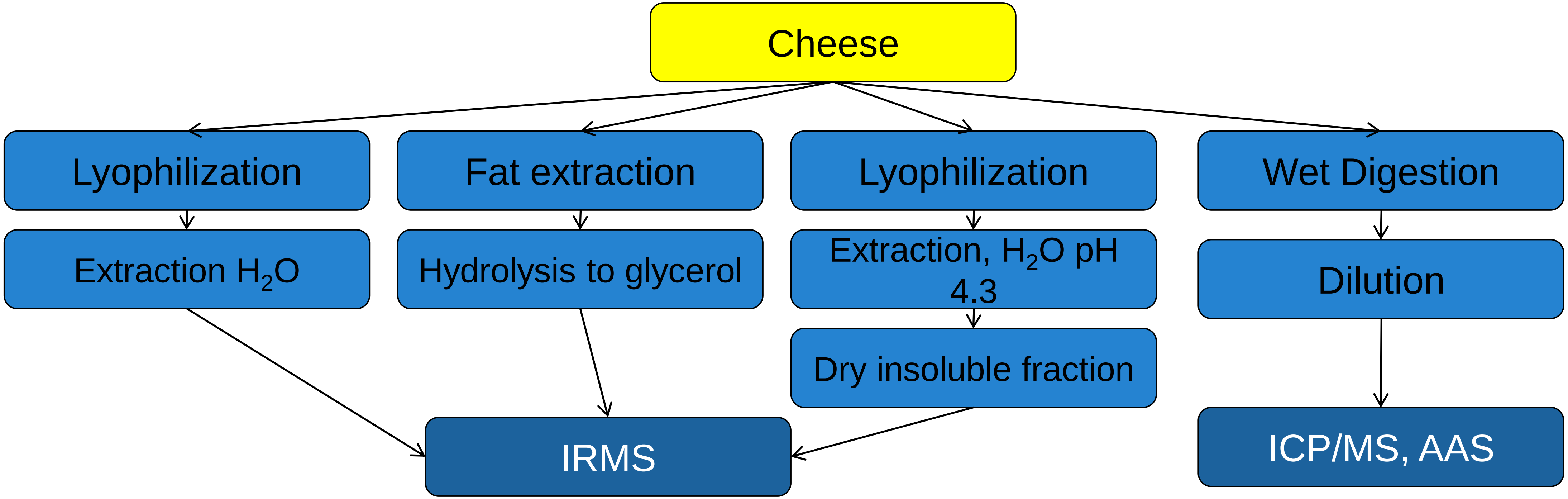


Honey 5

TMO appnot e30024

Assessing the origin of European cheeses

- Measurands taken: Stable isotope ratios, major, trace and radioactive elements
- Techniques used: IRMS, AAS, ICP-MS



Pillonel L et al, Lebensm.-Wiss. u.-Technol. 36 (2003) 615–623.

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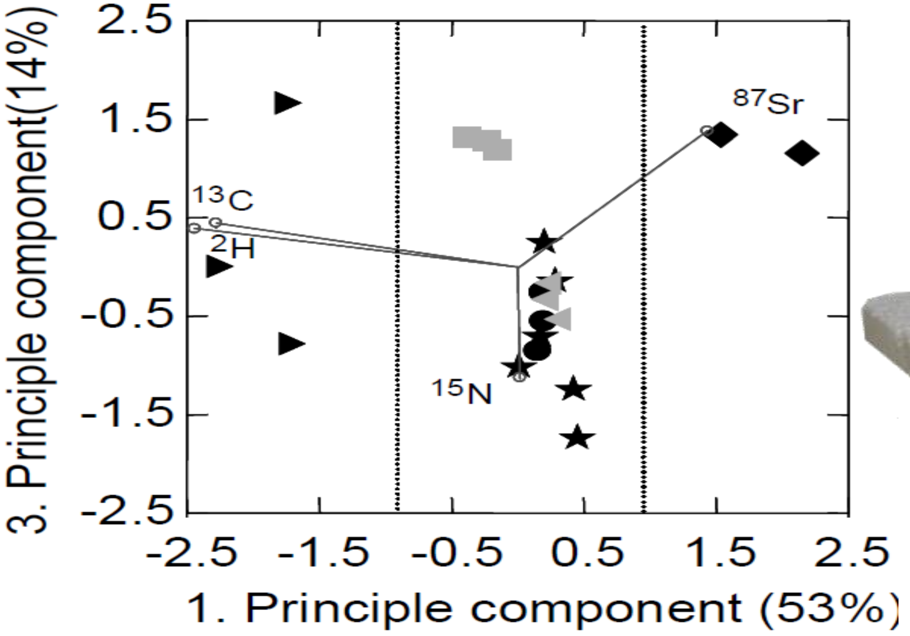
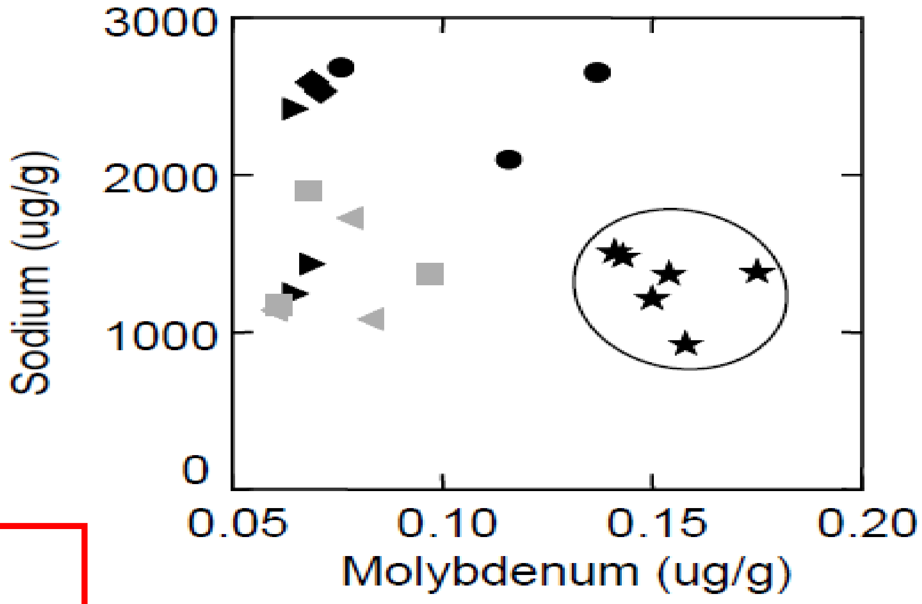
Assessing the cheese origin in Europe





Region

- ● Allgäu (D)
- ► Bretagne (F)
- ◆ Finland
- ■ Savoie (F)
- ★ Switzerland
- ◄ Vorarlberg (A)

Pillonel L et al, Lebensm.-Wiss. u.-Technol. 36 (2003) 615–623.

Infrared spectroscopy

- Benefits:
  - Ease of use
  - Fast response
  - No or limited sample preparation
  - Low price
- Limitations:
  - Lower sensitivity
  - Small spectral differences
- Typical examples: Near Infrared Spectroscopy (NIR, FT-NIR)
- Different Sampling accessories:
  - Sample cup spinner – heterogeneous materials
  - SabIR Fiber Optic Probe – material identification
  - Viscous Liquid Sampler – syrups, suspensions
  - Softgel detector (Gum)
  - Fiber Optic probes – in-process testing of solids and liquids

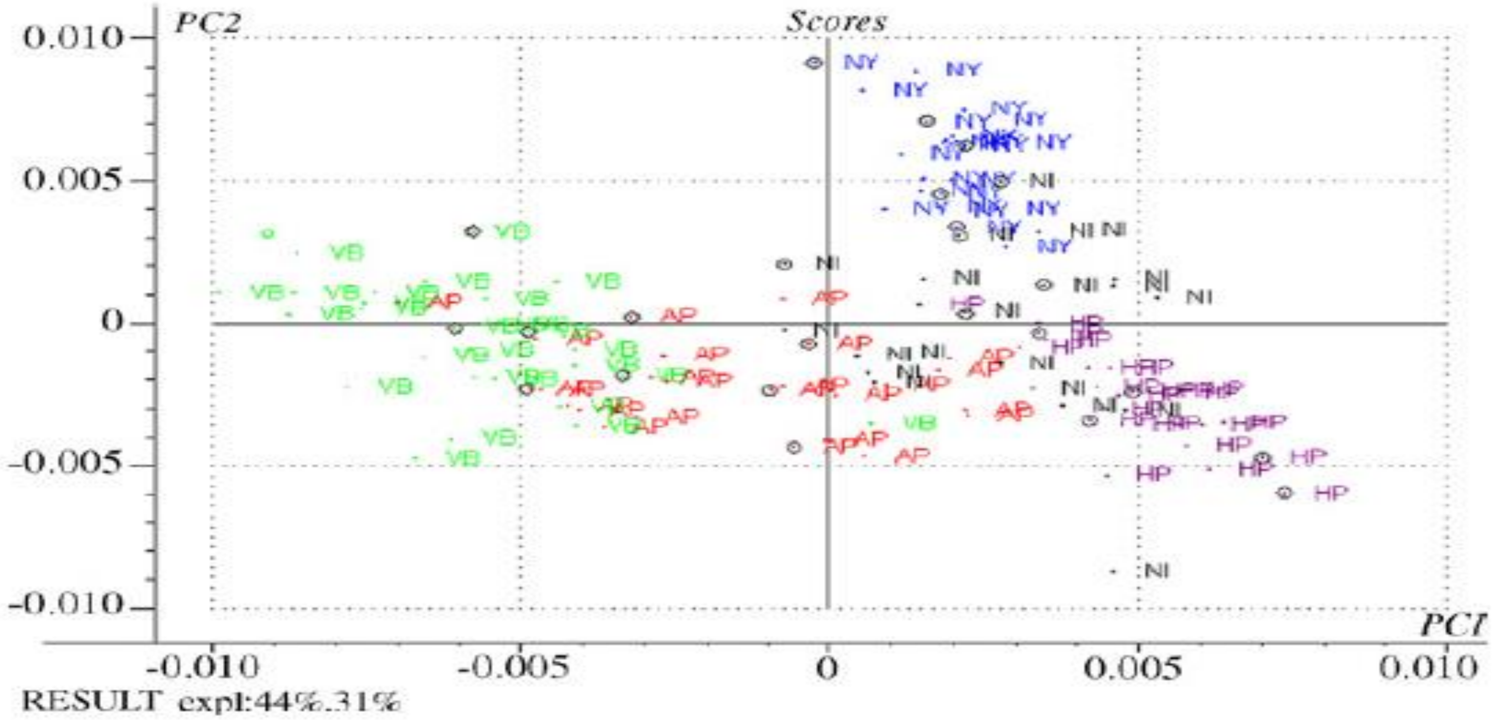
FT-NIR to assess geographic origin of olive oils

- Antaris FT-NIR with sample cup spinner
- Direct measurement in 2mm path length cell
- 4 cm^{-1} resolution
- Spectral range - 10000 and 4500 cm-1

O. Galtier et al.; Analytica Chimica Acta 595 (2007) 136–144.

FT-NIR to assess geographic origin of olive oils

- Quantification of fatty acids and triglycerols
- Classification in registered designation origin



O. Galtier et al.; Analytica Chimica Acta 595 (2007) 136–144.

What is the EU 2015/1006 regulation and how does it impact arsenic measurement in foods?

- It is an amendment to COMMISSION REGULATION (EC) No 1881/2006, which covers setting maximum levels for certain contaminants in foodstuffs
- REGULATION (EC) 1881/2006 includes inorganic Pb, Cd, Hg and Sn
- REGULATION (EU) 2015/1006 is the addition of inorganic As to the above list
- It's been developed because:
  - Inorganic arsenic shown to cause cancer (lung, bladder and skin) at exposures levels lower than previously thought
  - High consumers of rice most exposed to inorganic arsenic dietary exposure
  - Dietary exposure for children < 3 years old from rice-based foods is estimated to be about 2 to 3x that of adults

L 161/14 EN Official Journal of the European Union 26.6.2015

COMMISSION REGULATION (EU) 2015/1006

of 25 June 2015

amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs

(Text with EEA relevance)

What levels of inorganic arsenic need to be measured and in what samples?

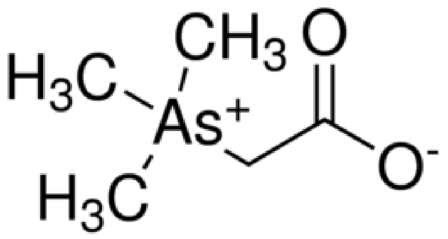
Arsenic (inorganic) ([50]) ([51])	Maximum levels (mg/kg wet weight)
Non-parboiled milled rice (polished or white rice)	0,20
Parboiled rice and husked rice	0,25
Rice waffles, rice wafers, rice crackers and rice cakes	0,30
Rice destined for the production of food for infants and young children ([3])	0,10

- The maximum levels of arsenic specified above apply from 1 January 2016
- The question is, if arsenic is present in your food, how much of it is actually inorganic arsenic?
  - **Just measuring total arsenic could give a false answer; food could be wasted that is actually safe to consume**
- How can you separate inorganic arsenic (As (III) and As (V)) from other arsenic species in the sample?
  - **By applying speciation using ion chromatography coupled to ICP-MS**

What is speciation?

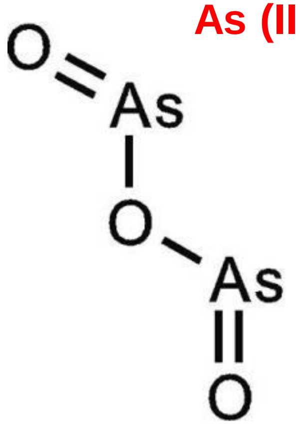
- **Definition** - ‘the analytical activity of identifying and/or measuring the quantities of one or more individual chemical species in a sample’ **IUPAC**
- **That means** – chemical species of an element are determined rather than the total element concentration
- **Example** – Arsenobetaine and As (III) species separated by ion chromatography (IC) and detected by ICP-MS

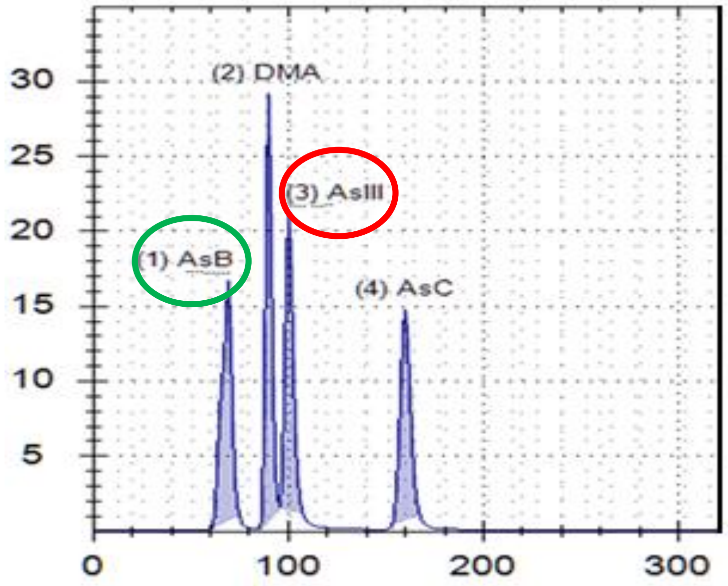
Arsenobetaine (AsB)



+

As (III)





Dimethylarsinic acid (DMA) and arsenocholine (AC) peaks also shown

- **Speciation analysis is important as it reveals valuable information about elemental bioavailability, mobility, metabolism and toxicity**

Speciation analysis using IC-ICP-MS

- Fully integrated hardware and software system:
  - Thermo Scientific™ Qtegra™ ISDS drives the whole system and includes:
  - Thermo Scientific Chromeleon™ plug-in drivers to control IC or HPLC
- Single control software
  - One software interface controls both the ICS and the ICP-MS
  - Includes all required functionality for fully automated data collection, calibration, quantitation and results reporting
  - Simple hardware connection – inert, metal free tubing connection from IC output to ICP-MS nebuliser

Thermo Scientific™ Dionex™ ICS-5000+

Thermo Scientific™ iCAP™ RQ

IC-ICP-MS is ideally suited to trace elemental speciation

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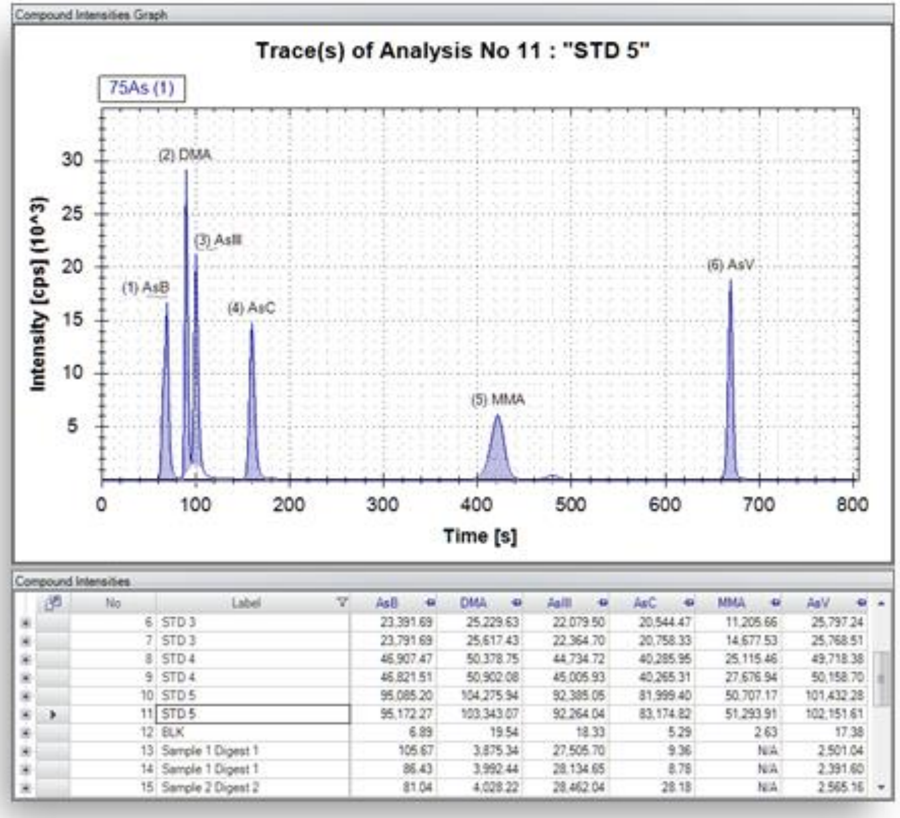
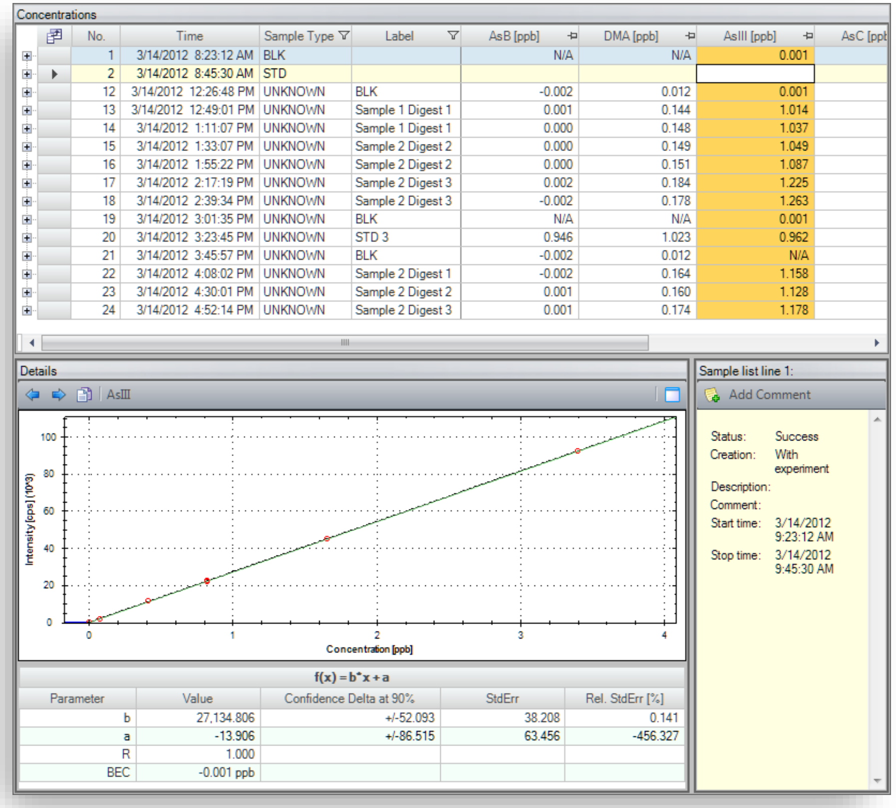
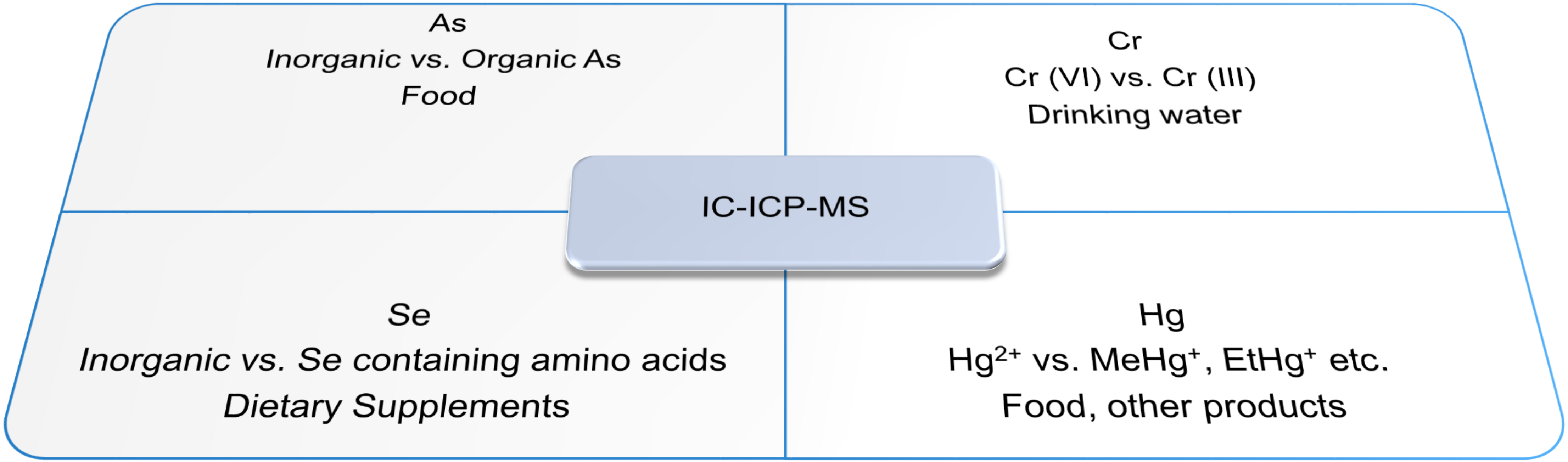
Qtegra ISDS: Full feature set for chromatography





Definition of compounds, retention times with automated peak detection.
Compound specific calibration graphs generated using the same workflow used in total element determinations

Overview of the range of IC-ICP-MS applications



Applications covered in this presentation:-

- Arsenic speciation in organic brown rice syrup
- Total arsenic quantification in food samples

Example application 2: Arsenic in organic brown rice syrup (OBRS)

- Rice is well known to accumulate As and can contain high amounts of this element

- Organic brown rice syrup is commonly used as a sweetener for toddler formulas and cereals
  - Healthier alternative to fructose containing corn syrups

- OBRS is also used in cereal bars and energy products for endurance athletes

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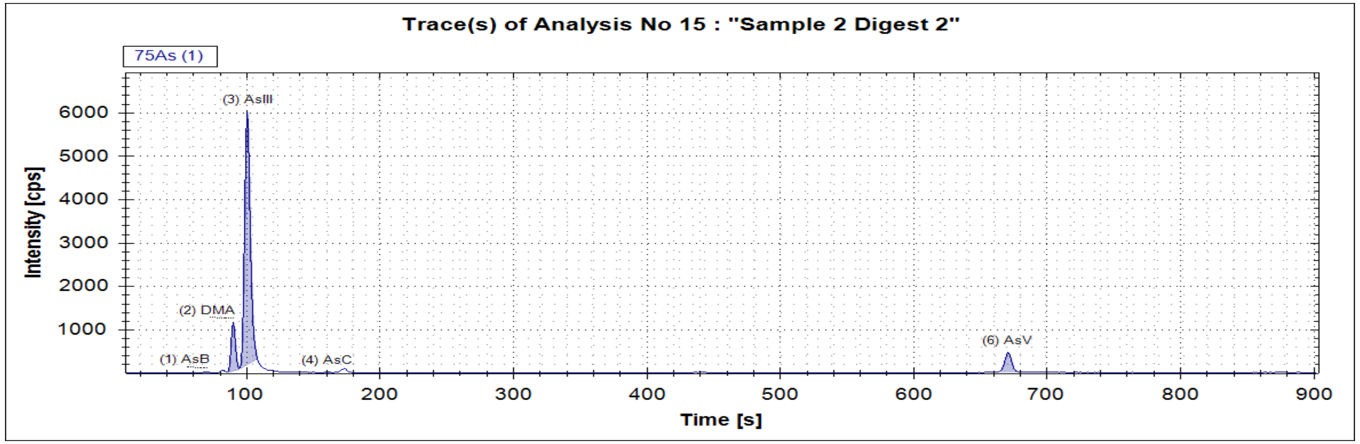
Example application 2: Arsenic in organic brown rice syrup (OBRS) - results

- Three different samples were sourced in Germany
- Total As determination: Closed vessel microwave digestion[1]
  - 0.5 g sample + 4 mL of 50% HNO_3, 10 minutes @ 180°C
  - Dilution to 20 mL total volume
  - Further dilution prior to measurement
- All samples contained ≥ 100 µg kg^{-1} As (which is the maximum level allowed in EU 2015/1006 for rice products for young children)
  - Sample 1: 118 ± 7 µg kg^{-1}
  - Sample 2: 136 ± 7 µg kg^{-1}
  - Sample 3: 107 ± 11 µg kg^{-1}
- Other studies have indicated that even higher As levels can be found in OBRS (between 80 - 400 µg kg^{-1})
- Speciation analysis was then conducted for all samples

1: Jackson, B. P. et al., Environmental Health Perspective, doi 10.1289/ehp.1104619

Example application 2: Arsenic in organic brown rice syrup (OBRS) - results

- Species extraction: Open vessel extraction[2]
  - 1.5 g sample + 15mL of 1% HNO_3, 90 minutes under reflux
  - Centrifugation + filtration
  - Dilution to 20 mL total volume + further dilution prior to measurement



Species distribution %	DMA	As III	As V	Sum
Sample 1	12%	82%	6%	100%
	12%	82%	6%	100%
	12%	81%	7%	100%
Sample 2	12%	83%	5%	100%
	12%	83%	5%	100%
	12%	82%	6%	100%
Sample 3	10%	84%	6%	100%
	10%	87%	3%	100%
	11%	86%	3%	100%

- Most abundant species was As (III), but As (V) was also present
- Most abundant organic As species was DMA
- Arsenobetaine was also detected
- Extraction efficiency was determined to be 74-78%

2: Huang et al., J. Anal. At. Spectrom. **25** (2010), 800-802

Example application 2: Total arsenic quantification in food samples

- Rice flour and chicken reference materials selected for this analysis
  - Rice Flour IRMM-804 and Chicken NCS ZC73016
- Multi-element determination, including As
  - Closed vessel microwave digestion
  - 0.5g of sample acid digested using a mixture of HNO_3 and HCl
- Reference materials analysed 5 times each during an extended run of 80 other digested food samples to test analysis repeatability
- Samples analysed using a Thermo Scientific™ iCAP™ RQ ICP-MS

Example application 3: Rice Flour IRMM-804 results

Isotope	Measured	Certified
^{55}Mn	35800 ± 470	34200 ± 2300
^{65}Cu	2650 ± 30	2740 ± 240
^{66}Zn	23100 ± 270	23100 ± 1900
^{75}As	52.3 ± 0.8	49 ± 4
^{78}Se	35.1 ± 1.0	38 (Reference value)
^{111}Cd	1620 ± 9	1610 ± 70
^{208}Pb	460 ± 8	420 ± 70

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Example application 3: Chicken NCS ZC73016 results

Isotope	Measured	Certified
^{7}Li	28 ± 1	34 ± 7
^{11}B	730 ± 23	760 ± 130
^{23}Na (ppm)	1310 ± 25	1440 ± 90
^{25}Mg (ppm)	1200 ± 22	1280 ± 100
^{31}P (ppm)	8950 ± 220	9600 ± 800
^{34}S (ppm)	8310 ± 220	8600 ± 500
^{39}K (ppm)	14000 ± 480	14600 ± 700
^{44}Ca (ppm)	200 ± 4	220 ± 20
^{52}Cr	450 ± 10	590 ± 110
^{55}Mn	1640 ± 20	1650 ± 70

Isotope	Measured	Certified
^{56}Fe	32700 ± 260	31300 ± 3000
^{60}Ni	153 ± 2	150 ± 30
^{65}Cu	1350 ± 11	1460 ± 120
^{66}Zn	25300 ± 220	26000 ± 1000
^{75}As	115 ± 1	109 ± 13
^{78}Se	549 ± 11	490 ± 60
^{88}Sr	611 ± 11	640 ± 80
^{98}Mo	112 ± 1	110 ± 10
^{138}Ba	1610 ± 16	1500 ± 400
^{208}Pb	90.7 ± 2.0	110 ± 20

Example application 3: Rice Flour IRMM-804 results – precision during the run (n=5)

Isotope	Mean	Standard deviation	% RSD
^{55}Mn	35500	170	0.5
^{65}Cu	2670	11	0.4
^{66}Zn	23400	170	0.7
^{75}As	52.7	0.7	1.4
^{78}Se	34.7	0.5	1.3
^{111}Cd	1630	11	0.7
^{208}Pb	490	3.9	0.8

Example application 3: Chicken NCS ZC73016 results – precision during the run (n=5)

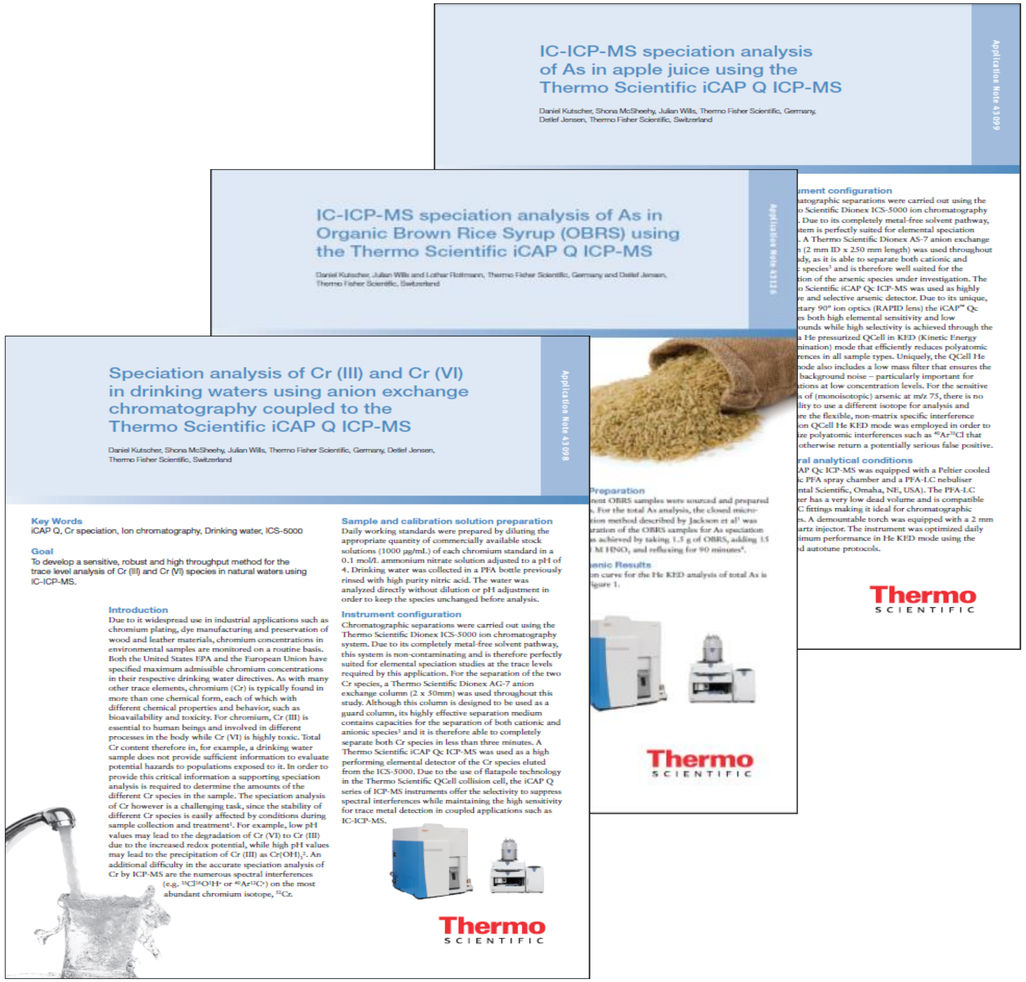
Isotope	Mean	Standard deviation	% RSD
^{7}Li	29	0.6	1.9
^{11}B	720	14	1.9
^{23}Na (ppm)	1380	18	1.3
^{25}Mg (ppm)	1170	13	1.1
^{31}P (ppm)	8700	150	1.7
^{34}S (ppm)	8230	150	1.9
^{39}K (ppm)	13800	240	1.8
^{44}Ca (ppm)	196	3.3	1.7
^{52}Cr	440	4.2	0.9
^{55}Mn	1630	13	0.8

Isotope	Mean	Standard deviation	% RSD
^{56}Fe	32600	225	0.7
^{60}Ni	149	1.2	0.8
^{65}Cu	1330	9	0.7
^{66}Zn	25200	150	0.6
^{75}As	114	1.0	0.9
^{78}Se	550	8.9	1.6
^{88}Sr	616	9.7	1.6
^{98}Mo	113	2.1	1.9
^{138}Ba	1620	23	1.4
^{208}Pb	89.8	0.9	1.0

Summary and conclusions

- Isotope Ratio Mass Spectrometry shown to be an ideal approach for food authenticity testing
  - Provides high isotope ratio accuracy and precision enabling clear identification of adulteration or provenance of food products
  - Covers a wide range of isotope groups – C, H, N, S and O
- Elemental analysis can provide additional information to IRMS
- Hyphenation of ion chromatography with ICP-Q-MS shown to be effective for speciation analysis of As in rice product samples
  - Excellent MDL obtained due to high sensitivity of iCAP Q
  - Narrow chromatographic peaks improve detection sensitivity
  - Low flow rates reduce sample- and mobile phase consumption
  - Ideal technique for meeting the demands of the EU COMMISSION REGULATION (EU) 2015/1006 relating to inorganic As in rice products
- Total arsenic quantification in food samples using ICP-MS demonstrated
  - Single analysis mode (He gas with kinetic energy discrimination) for multi-element analysis including As
  - Accurate and precise results obtained, with good internal standard robustness throughout the run

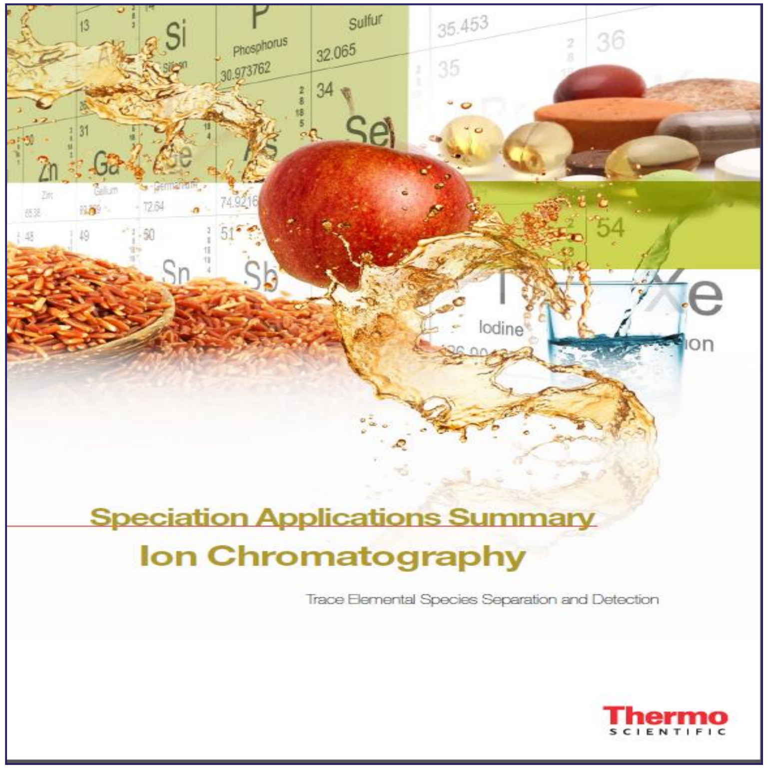
Available IC-ICP-MS application notes

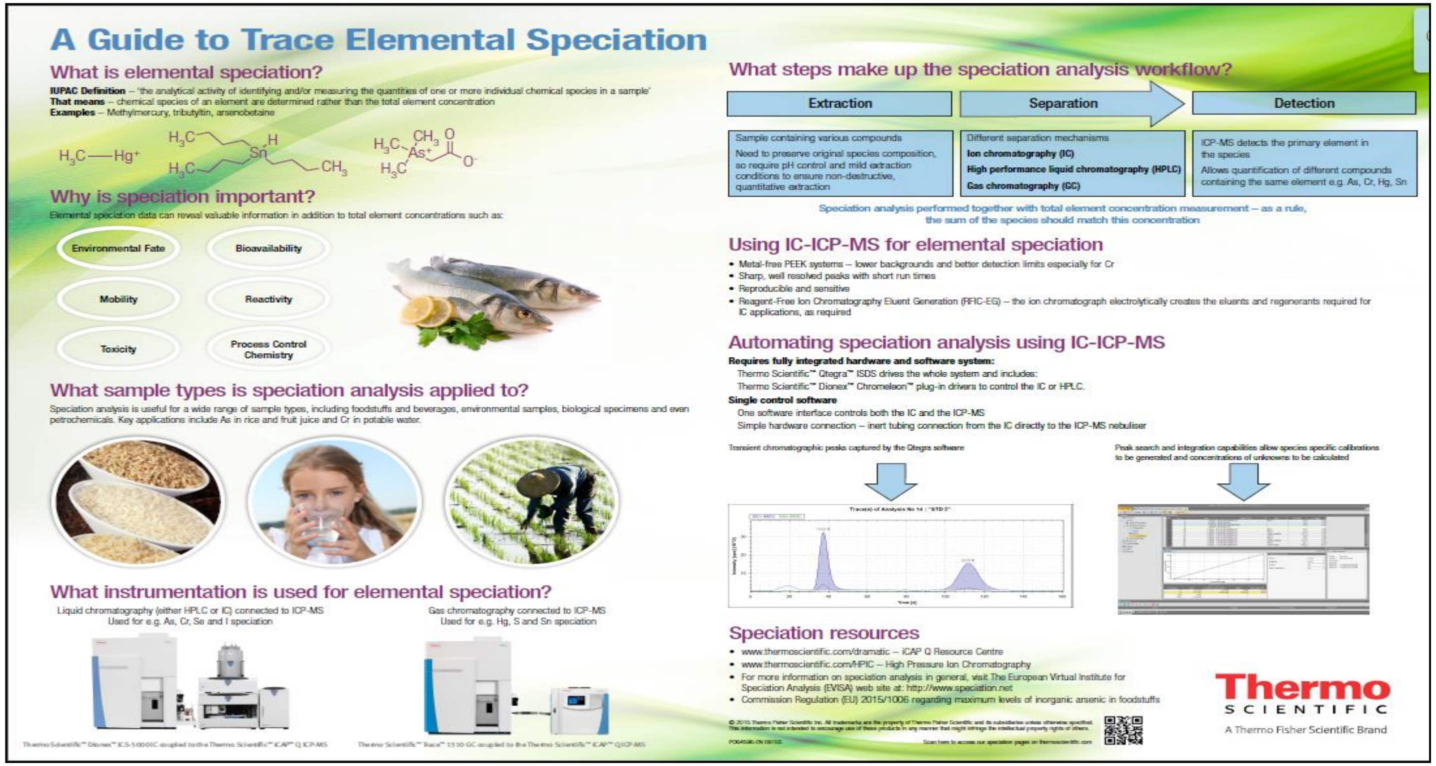


These notes and other useful information can be accessed by searching for ‘iCAP RQ (or iCAP Q) applications notes’ at **thermofisher.com**

Other resources

- AN 70590 Ion Chromatography Speciation Applications Summary
- PO 64596 TEA Speciation Poster





- Analyte Guru blog on the new EU legislation on arsenic in rice by Paul Dewsbury:

  http://analyteguru.com/arsenic-in-rice-new-legislation-comes-to-the-boil/