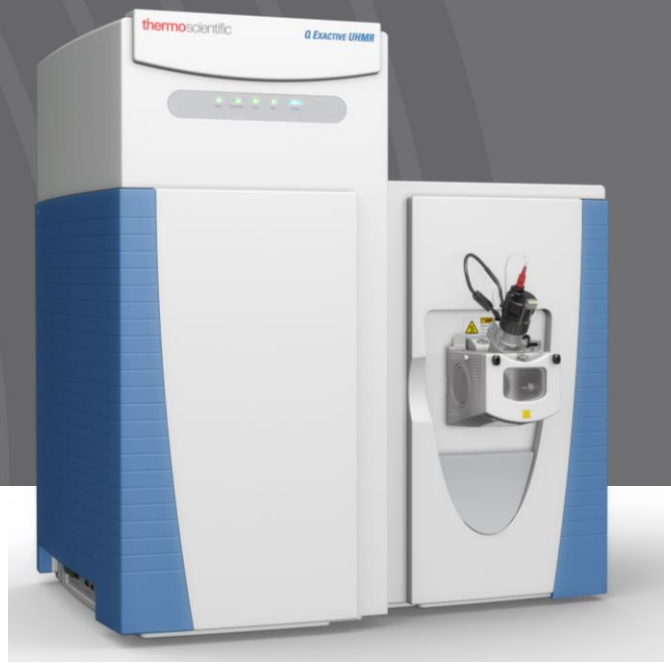


Total solutions for Food Safety Quality Assurance and Quality Control

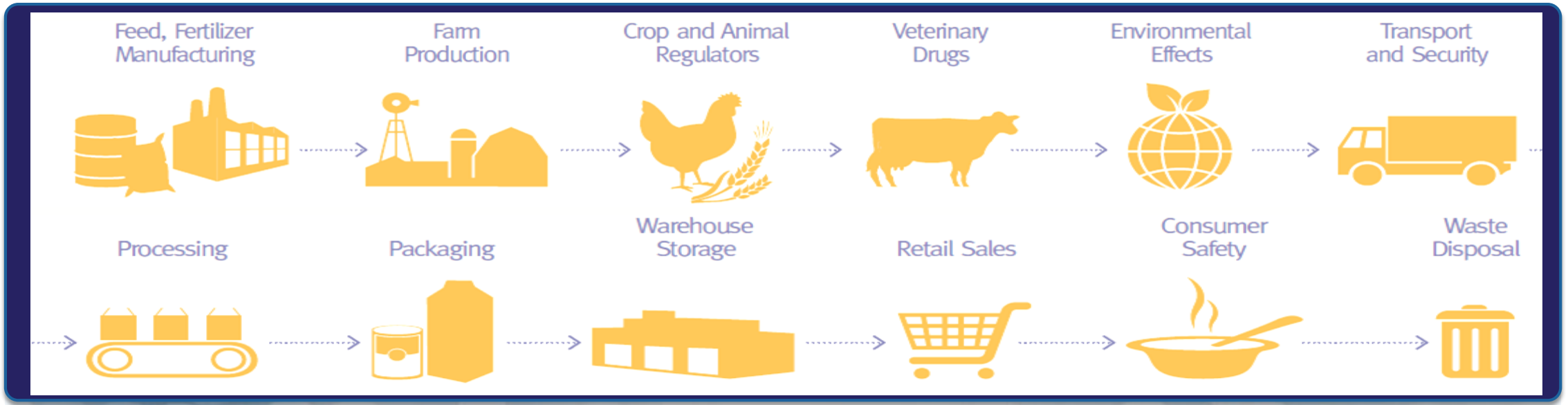
Dr John Xue

Agenda

- Quality and Risk Management of Food Supply Chain
- Food and Beverage Authenticity by Isotope Analysis
- Trace Elemental Analysis solutions
- Bacterial Proteotyping by HRMS

Contamination Threats to Food Supply Chain

Food supply chain from Farm to Fork



Major Types of contamination

Chemical *Biological* *Physical*

Contamination threats exist in each step of the food chain

ThermoFisher SCIENTIFIC

Our Unmatched Product Portfolio in Food Safety

No competitor can match the breadth and depth of our product portfolio

- Analytical Instruments
- Laboratory Equipment
- Laboratory Consumables and Chemicals
- Lab design, Furniture and Fume Hoods
- Microbiology Products
- Portable Analytical Instruments
- Product Processing Equipment
- Product Inspection Instrument
- LIMS and Laboratory Software
- Customer Channels and Services

Full capability to support customers in each stage of the food chain

Food Safety Testing Workflow (From Farm To Fork)

Sample Collection

- Tubes & Bottles
- Swabs
- Bags
- Gloves
- Vials

Sample Preparation

- Ovens
- Autoclave
- Water Purification System
- Centrifuge
- Biological Safety Cabinet
- Laboratory Refrigerators
- Weighing Balances
- Homogenizers
- Concentrators
- Water Baths
- Enrichment Media & Plates
- Pipettes and Tips
- pH Meter
- Label Printers
- Shakers
- Automated IMS
- DNA purification kit
- Dynabeads
- Automated Sample Preparation Solutions
- Automated Extraction

Sample Analysis

Microbiological Testing

- Biochemical & Serological Identification Test
- Dehydrated culture media
- Prepoured media
- Bagged media
- Colony Counter
- Incubators
- Microscopy

DNA/ Allergen Testing

- Liquid Chromatography Mass Spectrometry (LC-MS)
- GMO, Halal, Meat Identification
- Gel Documentation Imagers
- PCR & qPCR
- Microplate reader
- Pathogen test kit
- Sanger/NGS

Food Processing/ Nutrition/ Pollutant Testing

- Chromatography and Mass Spectrometry System
- Elemental Analysis (organic and inorganic)
- Discrete Analyzer
- Portable Analyzers
- Photometric system
- Chemicals & Consumables

Sample Storage/ Data Management

- Ultra-low Temperature Freezers
- Wireless Monitoring System
- Chromatography Data System
- Lab Information Management System

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Food Safety Testing Workflow (CMD)

Sample Preparation → **Sample Analysis** → **Data Management**

AUTOMATED SAMPLE PREPARATION

Accelerated Solvent Extraction (ASE)

Automated Solid-Phase Extraction

Automated Solvent Evaporation

DNA/ALLERGEN TESTING

Isotope Ratio Mass Spectrometry (IRMS)

Liquid Chromatography Mass Spectrometry (LC-MS)

Liquid Chromatography High Resolution Accurate Mass Spectrometry (HRAM)

FOOD PROCESSING/ POLLUTANT TESTING

Ion Chromatography (IC)

Chromatography (Liquid or Gas)

Mass Spectrometry (MS)

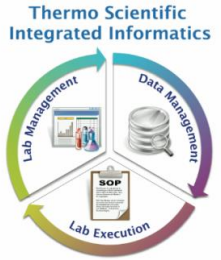
Trace Elemental Analysis **(TEA)**

Liquid Chromatography High Resolution Accurate Mass Spectrometry (HRAM)

DATA MANAGEMENT

Chromatography Data System (CDS)

Laboratory Information Management System (LIMS)



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Quality and Risk Management

Self-control
(comparing charges, raw materials and endproducts)

External-control
(suppliers, competitors)

Analytical protection
(assistance of audits, marketing-advantage)

No-paper based verification
(based on stored reference samples)

Brand-protection
(through „labeling“ using isotope-tracers for fraud-protection)

Demand from the Industry

Quality Control – Quality Management

Traceability

Brand Protection

...

Marketing, Public Awareness

Comparative Advantage

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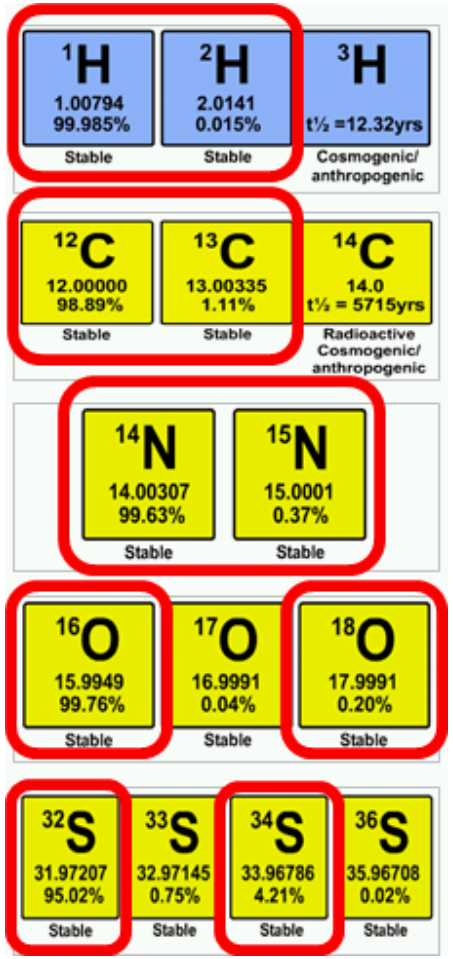
Some Examples of Food Fraud

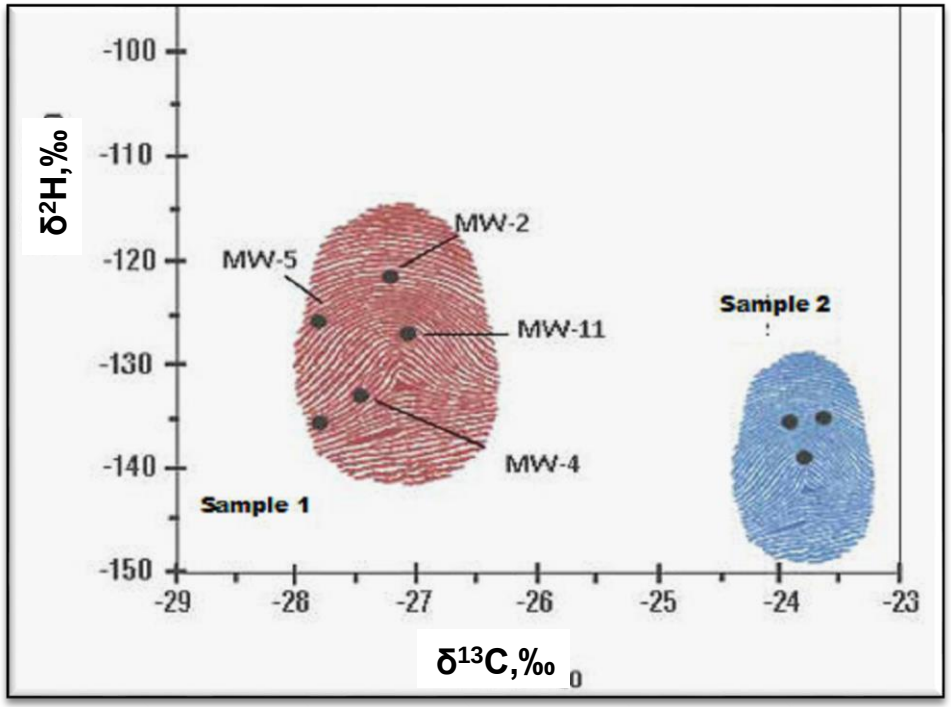
Food and Beverages:	Potential Fraud:
Fruit juices	Watering, sweetening
Wine	Watering, chaptalization, label declaration
Vinegar	Origin identification (maize, cider, grape, …)
Beers	Origin identification (grains other than malt)
Alcoholic beverages	Mislabeling, origin identification
Honey	Addition of inverted and cane sugars
Olive oils	Addition of cheaper oils
Tea, Coffee	Mislabeling and origin
Dairy products	Addition of undeclared milk, Mislabeling
Meat	Mislabeling (origin) and feeding diet
Fish	Mislabeling (wild ↔ farmed)
Fruit and vegetables	Mislabeling (organic versus inorganic)

5 organic elements

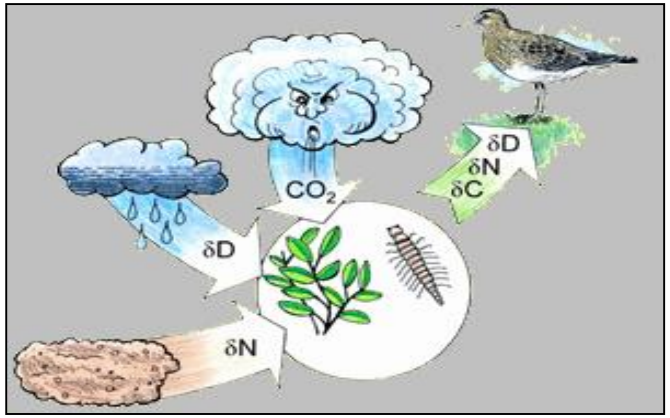
$^{2}H/^{1}H, ^{13}C/^{12}C, ^{15}N/^{14}N, ^{18}O/^{16}O, ^{34}S/^{32}S$



chem./physical. processes



influence factors on isotope ratios

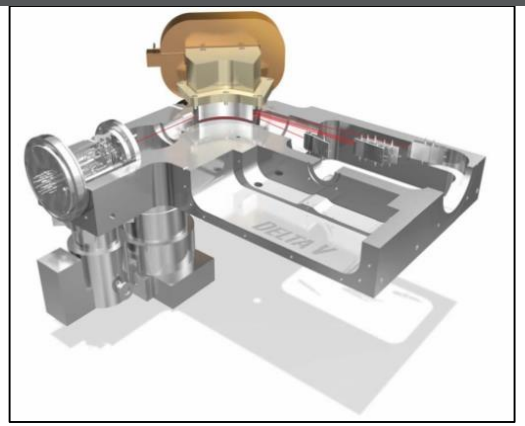


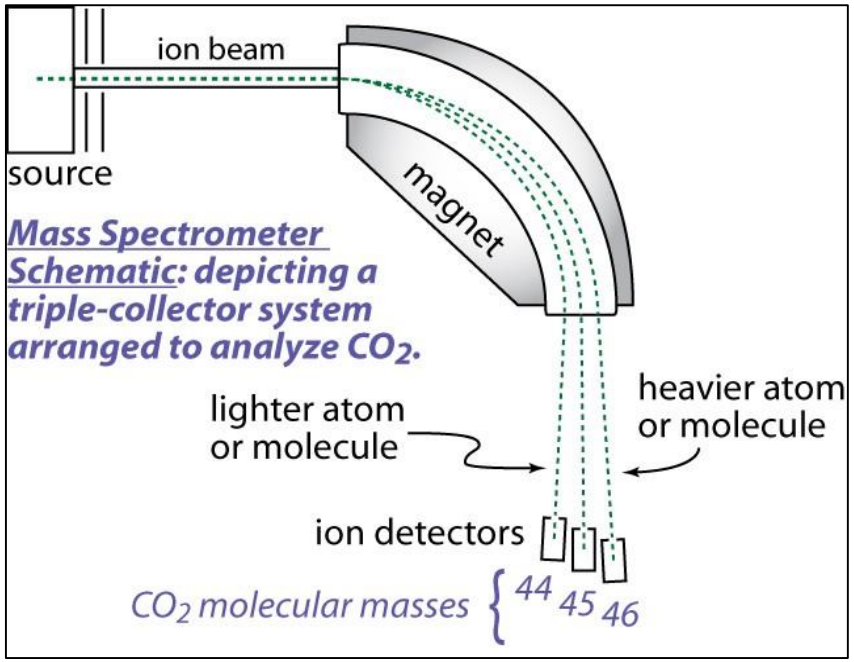
isotope-fingerprint

measured with IRMS (Isotope Ratio Mass Spectrometry)

Principles of Isotope Analysis

Thermo Scientific™ Flash™ 2000 Elemental Analyser connected to a Thermo Scientific™ DELTA™ V Isotope Ratio Mass Spectrometer





http://serc.carleton.edu/research_education/geochemsheets/techniques/gassourcemassspec.html

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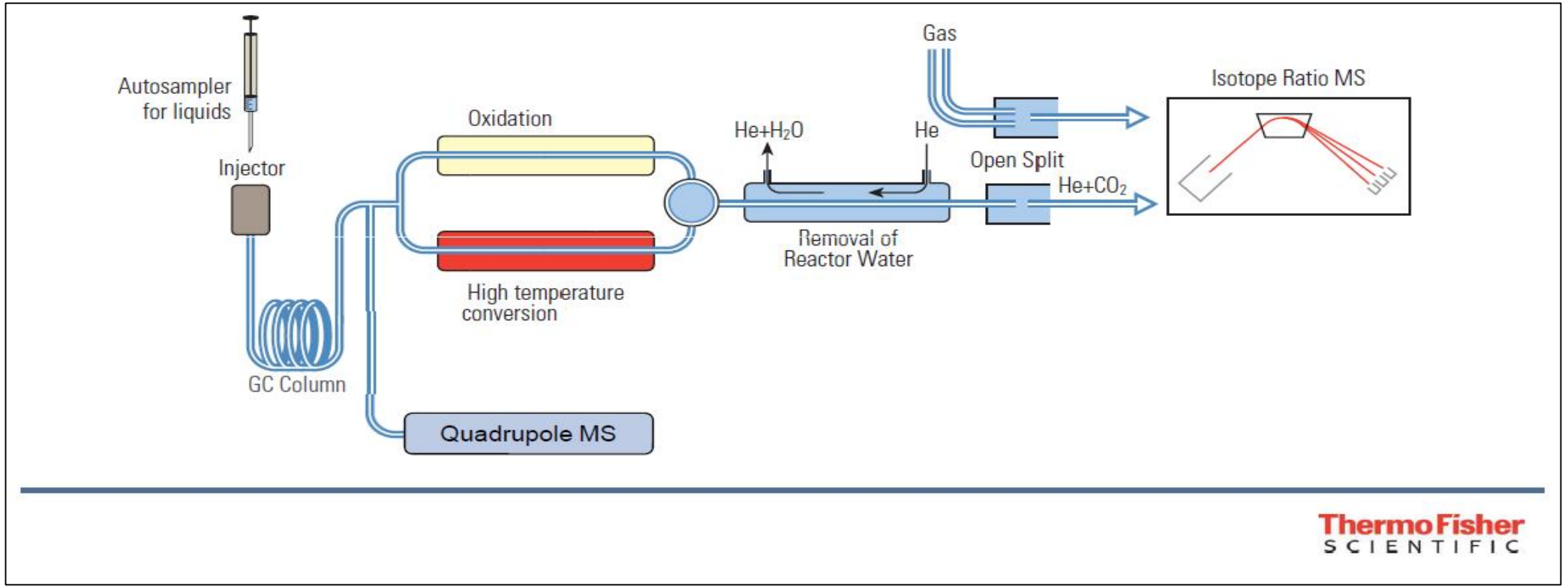
Compound Specific Isotope Analysis (CSIA)
coupling a GC to the IRMS via oxidation or high temperature conversion tubes



Authenticity of Fruit Juices

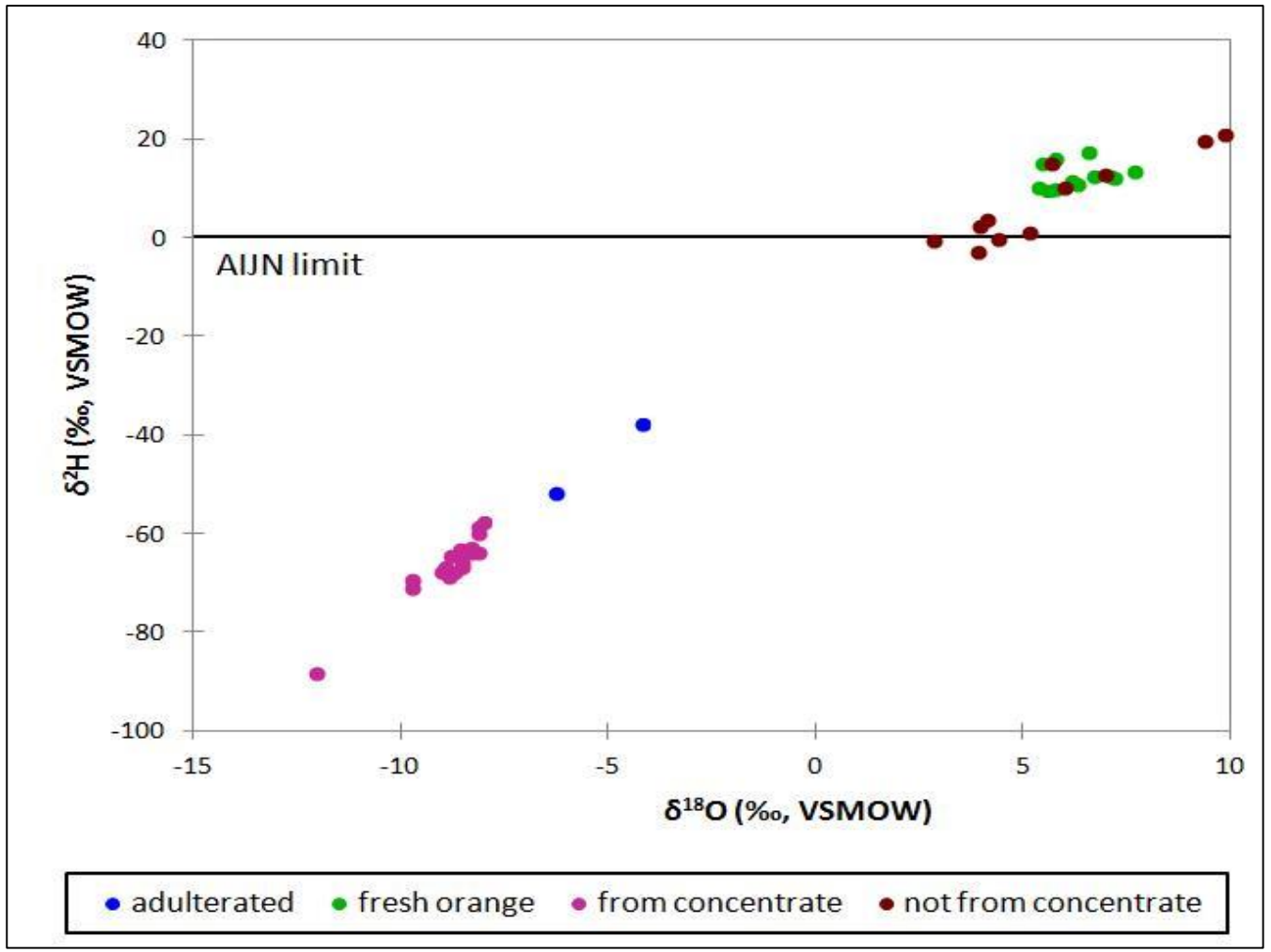
Quelle: www.wissen.de

Quelle: www.fitforfun.de

- **Differentiation of direct juice and juice from concentrate**
- **Detection of water, organ acids**
- **Detection of added sugar**
- **Origin verification of fruits**

Authenticity of Fruit Juices

Verification of fresh orange juices (NFC)



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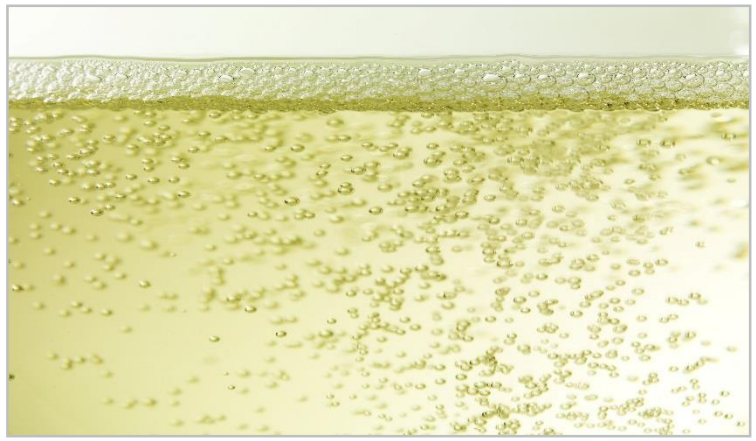
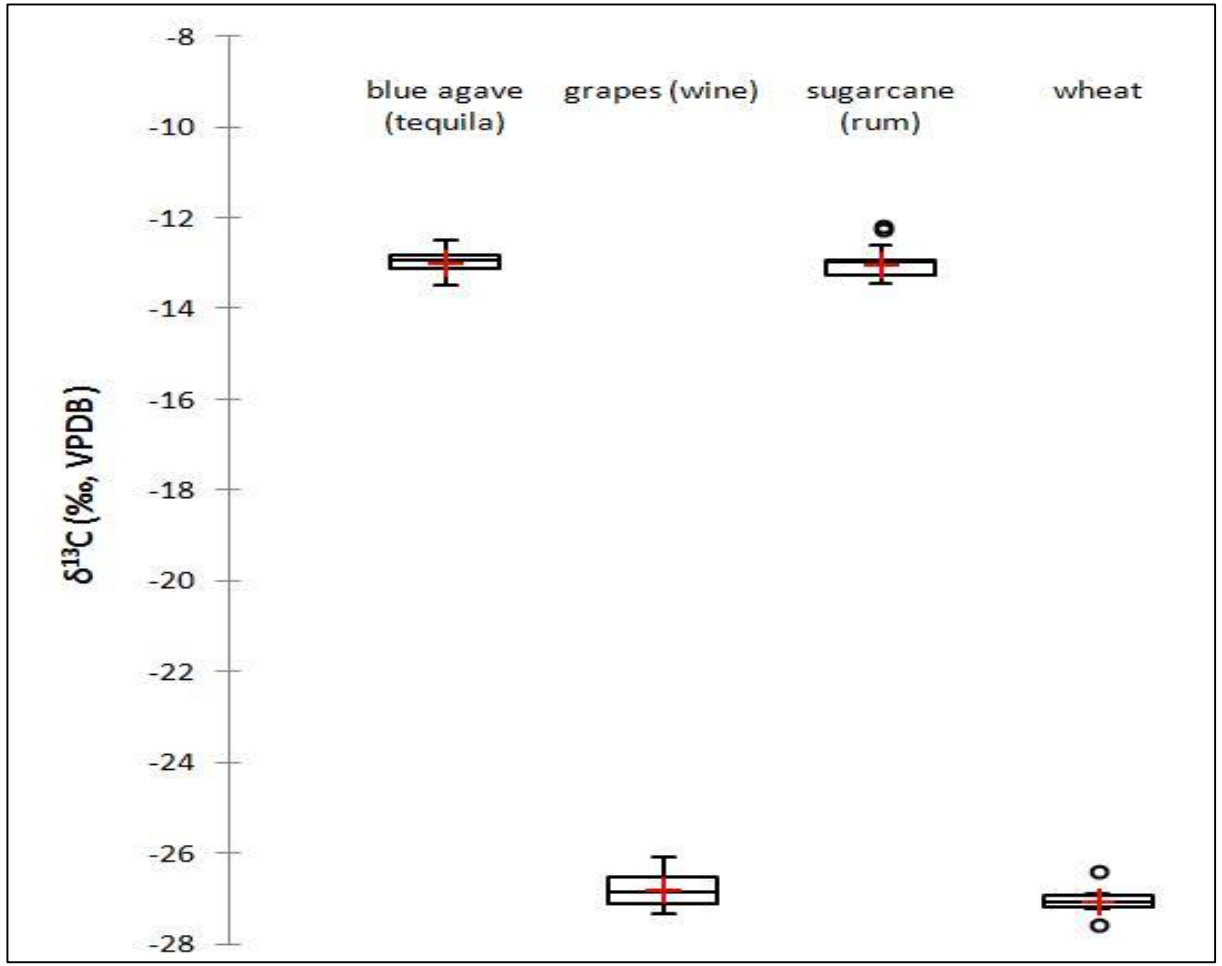
Authenticity of Wine / Sparkling Wine / Beer



Verification of alcohol source



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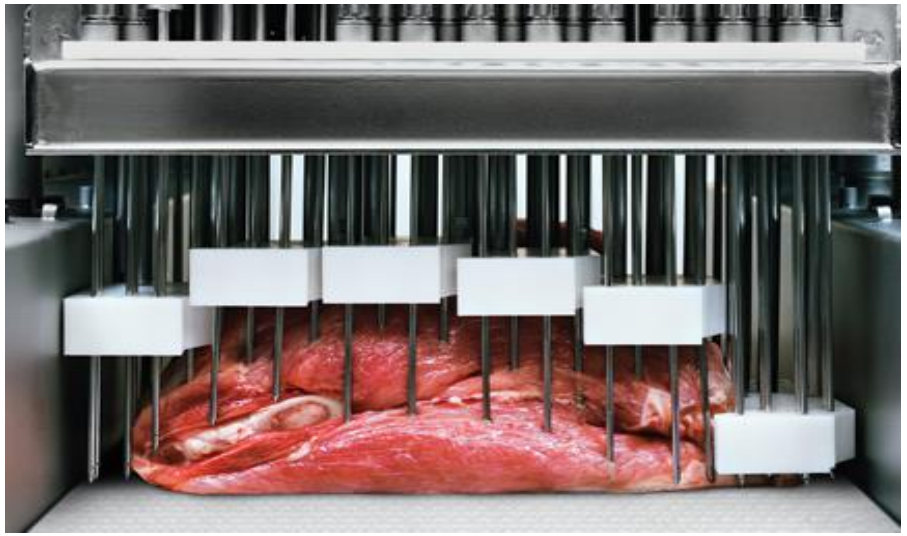
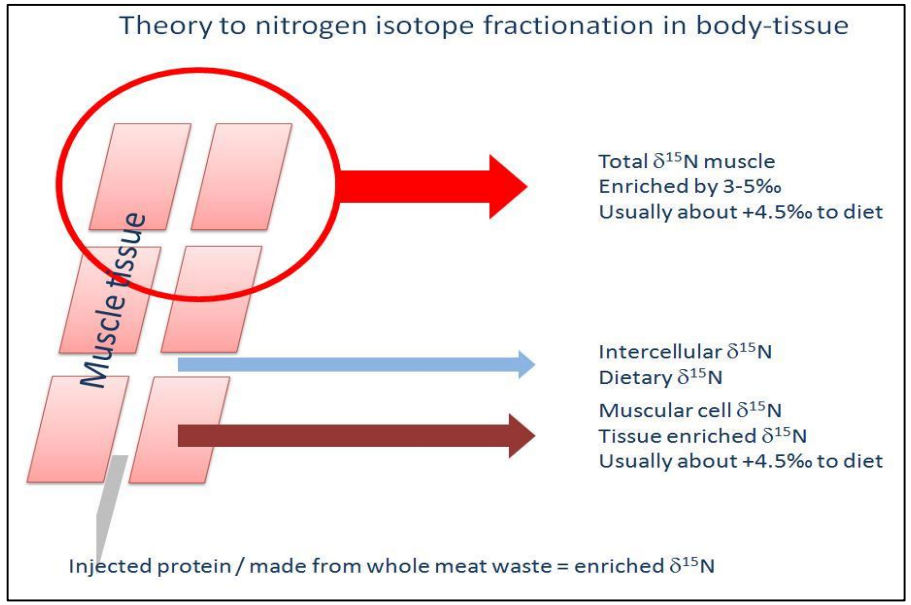
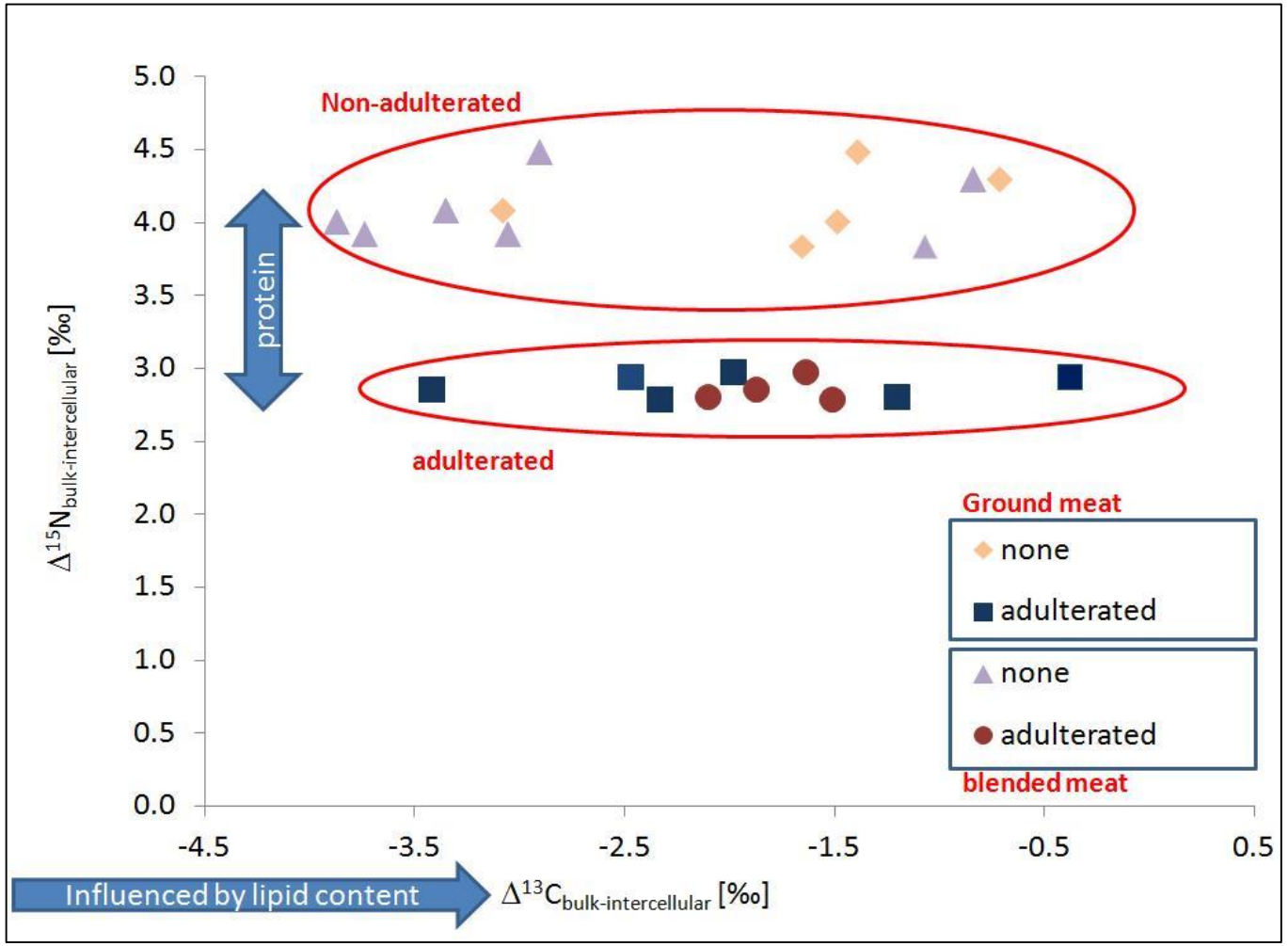
Food Additives – It Get‘s Complicated





Injection of pork protein into pork meat



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EA-IRMS: δ^2H and $\delta^{18}O$ in Roasted Coffee Beans

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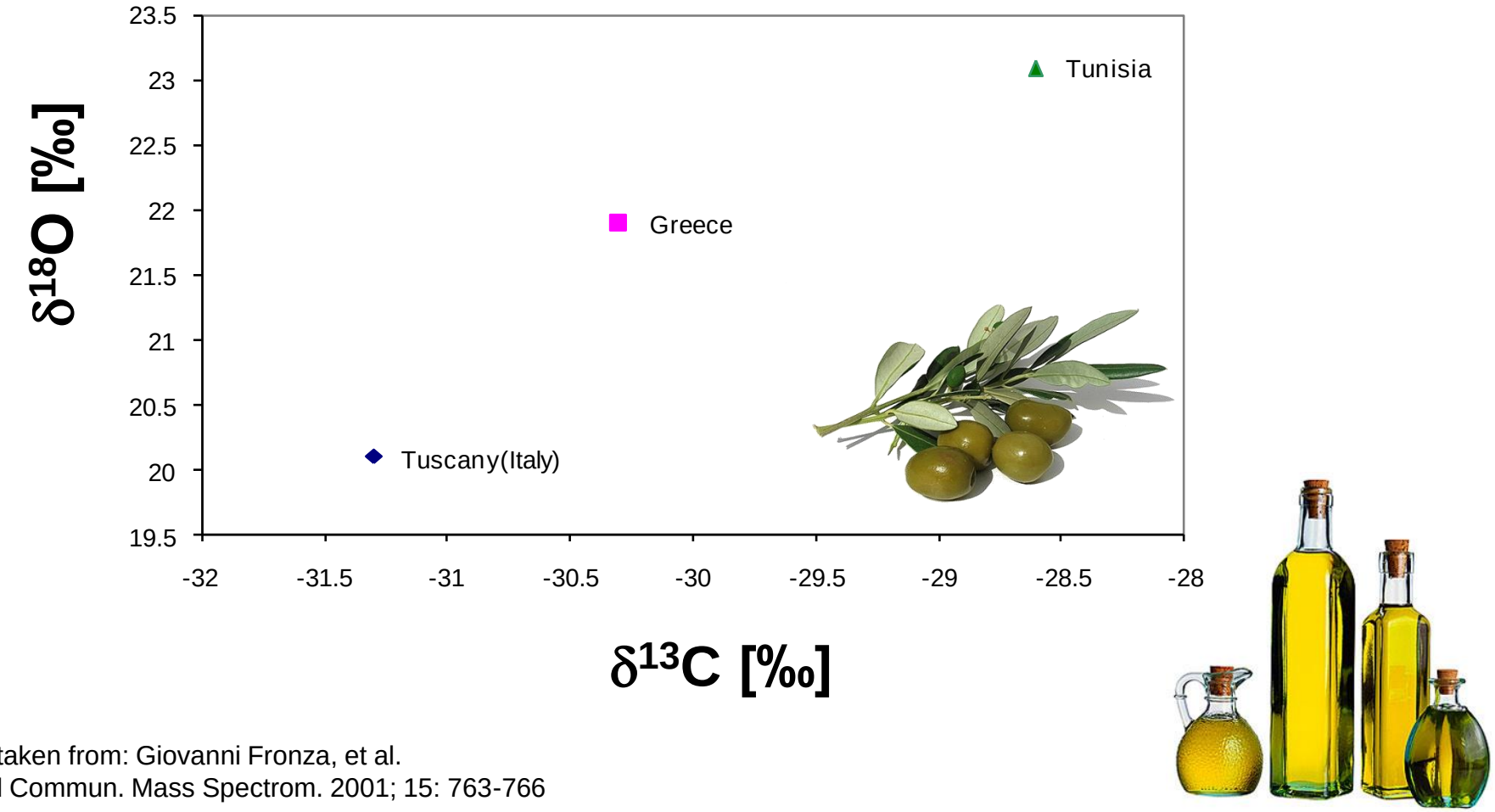
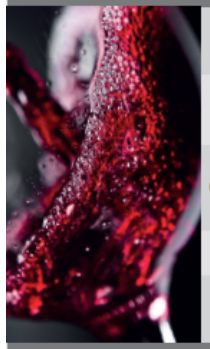
EA-IRMS: $\delta^{13}C$ and $\delta^{18}O$ in Olive Oil

Where does your olive oil come from?



Data taken from: Giovanni Fronza, et al.
Rapid Commun. Mass Spectrom. 2001; 15: 763-766

Official methods and Isotope Fingerprints

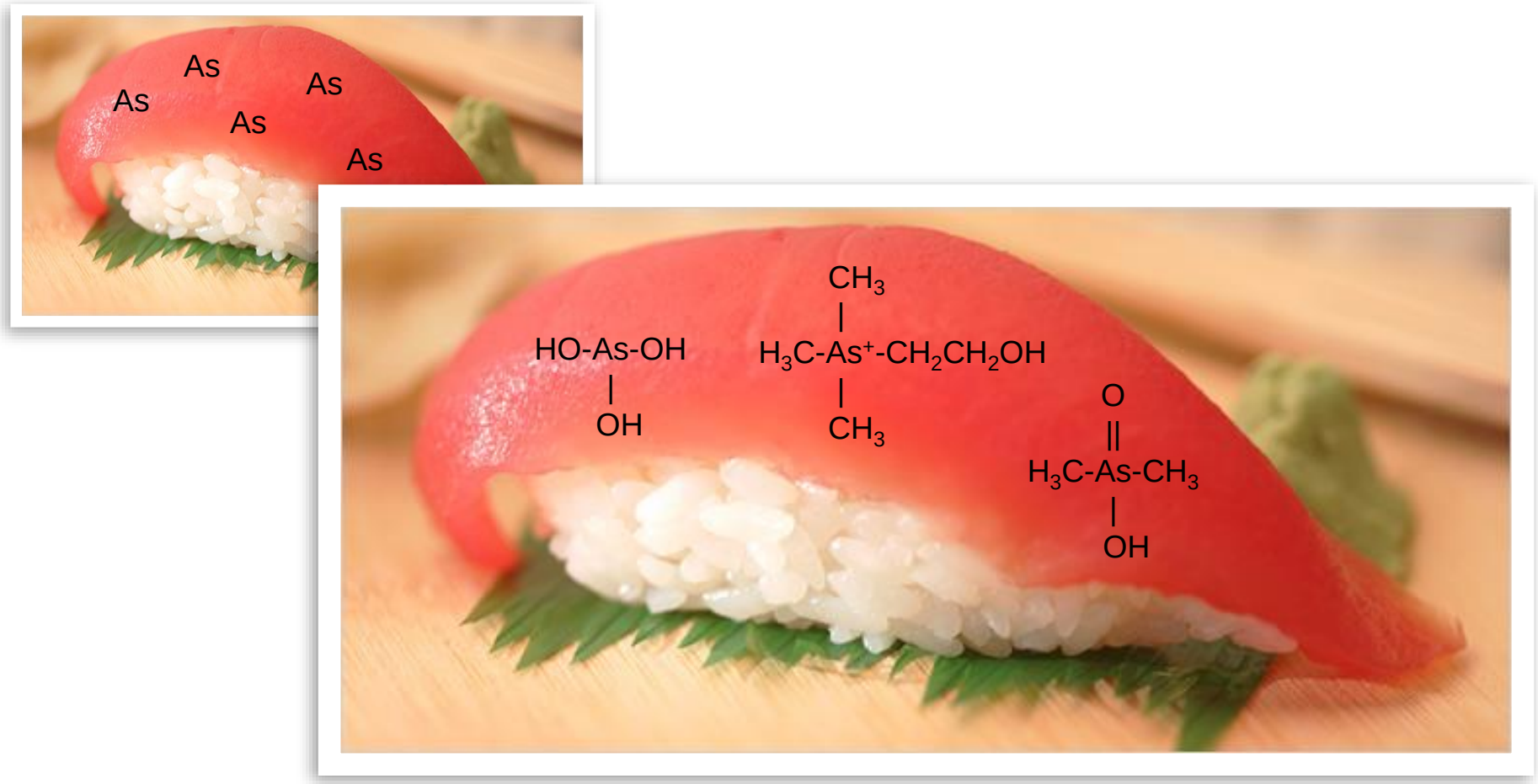
Product	Official method	Isotope fingerprint	Sample	What does it address?	Analytical solution
Wine					
	OIV-MA-AS2-12	$\delta^{18}O$	Water	Adulteration, Geographical origin, Year of vintage	Thermo Scientific™ GasBench II System, Thermo Scientific™ Dual Inlet
	OIV-MA-AS312-06	$\delta^{13}C$	Ethanol, Wine must, Grape sugar	Adulteration, origin	Thermo Scientific™ EA IsoLink™ IRMS System, Thermo Scientific™ GC IsoLink II™ Interface for GC-IRMS
	OIV-AS312-07	$\delta^{13}C$	Glycerol in wines	Adulteration by addition of glycerol from C4 maize or Fossil sources	GC IsoLink II Interface for GC-IRMS, Thermo Scientific™ LC IsoLink™ Interface for IRM-LC/MS
	OIV-OENO 510-2013	$\delta^{13}C$	Acetic acid in wine, vinegar		GC IsoLink II Interface for GC-IRMS, EA IsoLink IRMS System
	OIV-OENO 510-2013	$\delta^{18}O$	Water in wine, vinegar	Adulteration, Geographical Origin, Year of Vintage	Thermo Scientific™ GasBench II System, Dual Inlet
Sparkling wine					
	OIV-MA-AS314-03	$\delta^{13}C$	CO_2 in sparkling wine	Origin and authenticity of sparkling wine	GasBench II System, EA IsoLink IRMS System, GC IsoLink, Dual Inlet
Spirits					
	OIV-AS312-07	$\delta^{13}C$	Glycerol in spirits	Adulteration by addition of glycerol from C4 maize or Fossil sources	GC IsoLink II Interface for GC-IRMS, LC IsoLink Interface for IRM-LC/MS
Fruit Juice					
	EU – CEN 1995	$\delta^{13}C$	Sugars	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface
	USA – AOAC 1981	$\delta^{13}C$	Sugars	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface
	EU – CEN 1998	$\delta^{13}C$	Sugars and pulp	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface
	EU – CEN 1995	$\delta^{2}H$ and $\delta^{18}O$	Water	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface
	AOAC method 2004.01	$\delta^{13}C$	Ethanol (From Fermentation)	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface
Fruit Juice (Concentrate)					
	AOAC 1992	$\delta^{18}O$	Water	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, EA IsoLink IRMS System
Honey					
	AOAC method 991.41	$\delta^{13}C$	C-4 plant sugars at concentration >7%	Adulteration of honey	EA IsoLink IRMS System
	AOAC method 998.12	$\delta^{13}C$	C-4 plant sugars at concentration >7%	Adulteration of honey	EA IsoLink IRMS System
Cheese					
	EU Reg 548/2011	$\delta^{13}C$	PDO	PDO Grana Padano	EA IsoLink IRMS System

Which One Is Safer to Eat?

Total As concentration is not enough

Elemental Speciation Analysis

Separation and quantification of different chemical forms of an element to understand environmental or health related impacts associated with a sample



Who Needs to Perform Speciation Analysis?

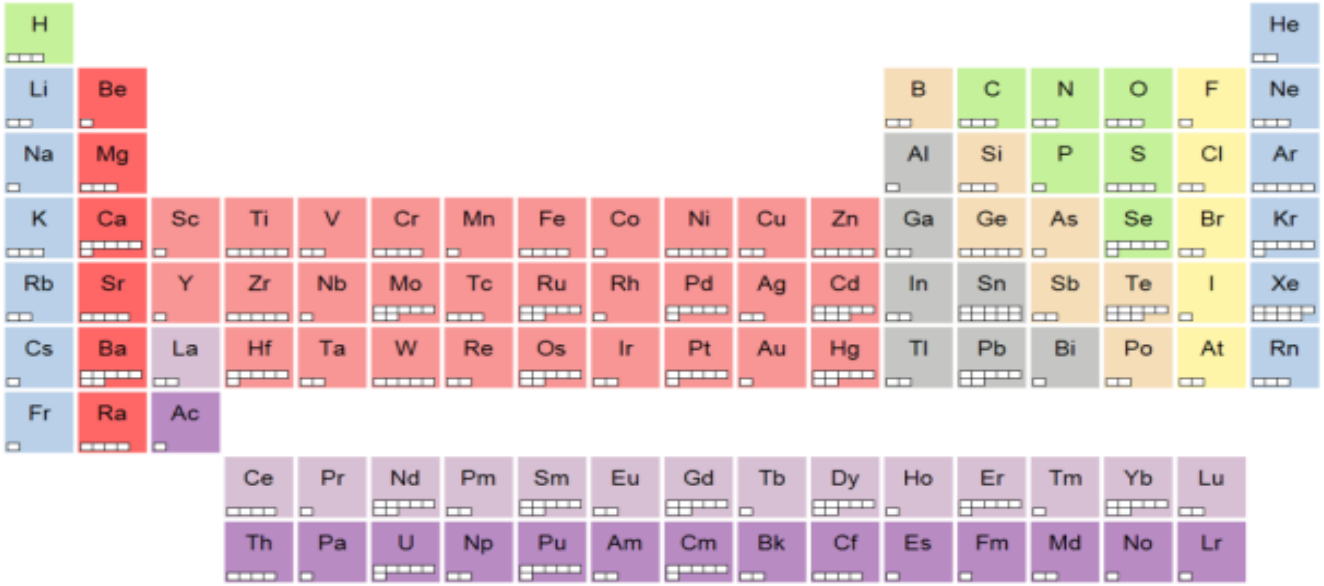
Industries	Applications
Environmental	• Hexavalent chromium, arsenic and bromate in drinking waters
Food Safety	• Arsenic in fruit juices and rice grains • Mercury in fish
Occupational Exposure and Consumer Goods	• Hexavalent chromium in toy materials
Pharmaceutical	• Mercury in herbal supplements
Petrochemical	• Sulfur/selenium in produced water
Electrical Production	• Selenium in wastewater

Regulations

More regulations to come with ongoing assessments

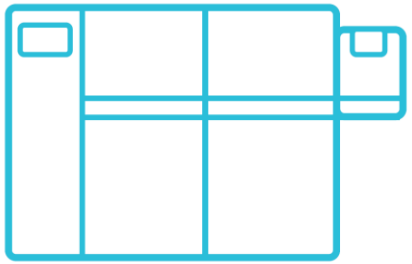
Advantages of ICP-MS

- Measures almost the whole periodic table in any matrix
  - Elemental concentrations
  - High precision isotope ratio determinations
  - **Species information** when coupled to separation devices



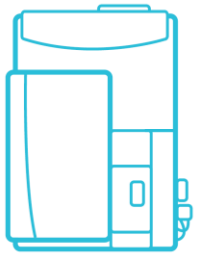
Highly versatile and sensitive detection technique

ICP-MS Performance for All Applications



Thermo Scientific ELEMENT 2/XR HR-ICP-MS
High Resolution ICP-MS

Thermo Scientific iCAP TQ ICP-MS
Triple quadrupole ICP-MS



Thermo Scientific iCAP RQ ICP-MS
Single quadrupole ICP-MS

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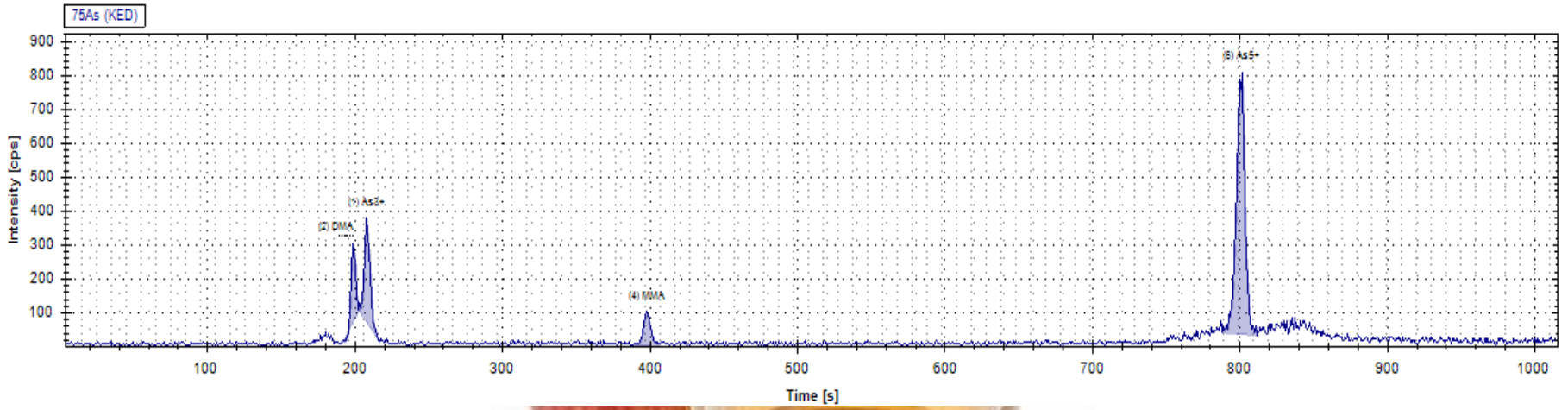
Arsenic Speciation in Apple Juice



	DMA [ng g^{-1}]	As (III) [ng g^{-1}]	MMA [ng g^{-1}]	As(V) [ng g^{-1}]	Sum [ng g^{-1}]	Total As [ng g^{-1}]
Juice 3	-	0.5 ± 0.01	-	0.8 ± 0.01	1.3	1.7 ± 0.05
Juice 4	0.4 ± 0.05	0.3 ± 0.01	0.1 ± 0.05	0.7 ± 0.01	1.5	1.8 ± 0.05

Sensitive detection down to ppt levels

- Not detected

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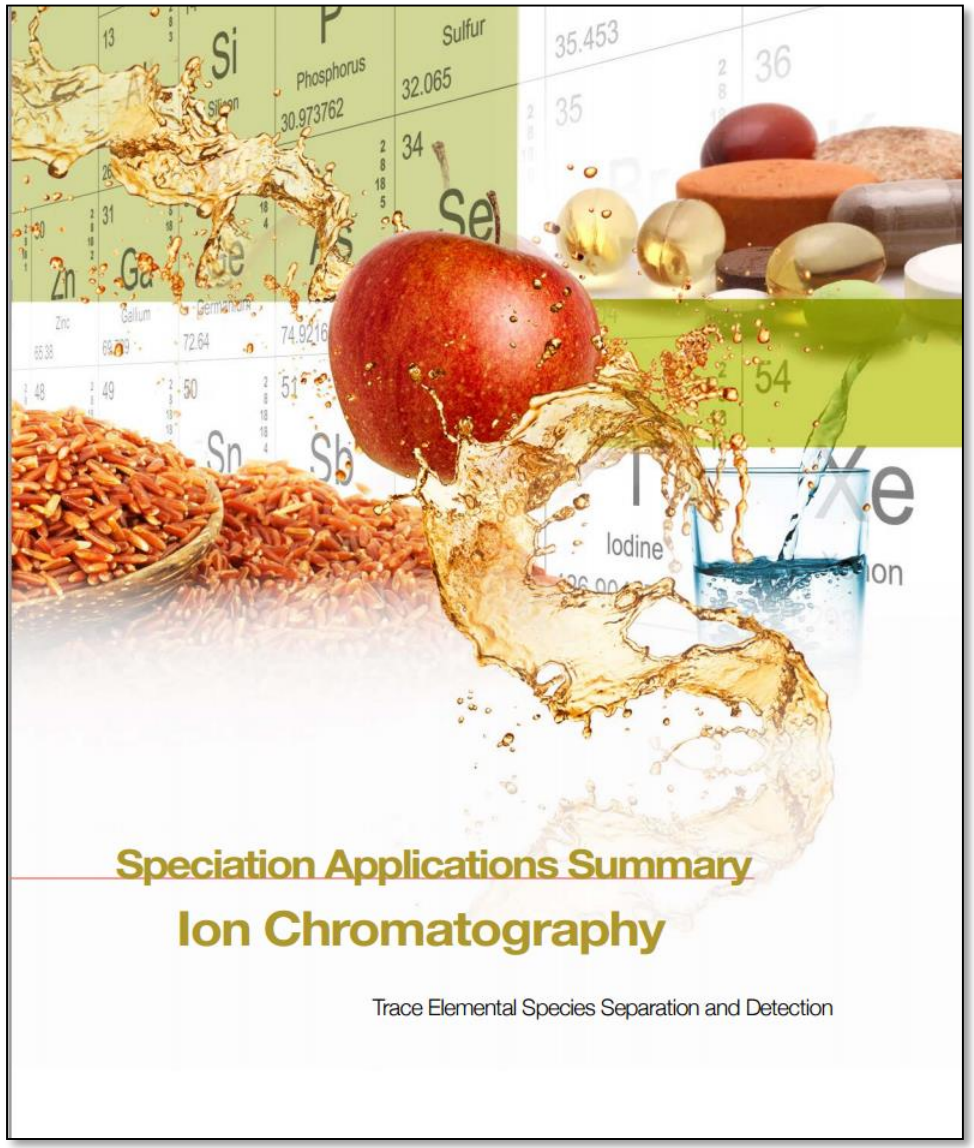
Collateral: Speciation Applications Summary



Compilation of methods for:
- Arsenic
- Chromium
- Iodide
- Mercury

Download here

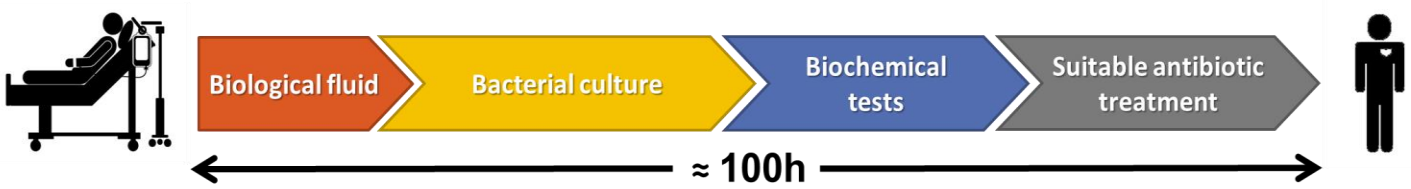
Update in progress
- *Arsenic in Rice*
- *Bromate in DW*
- *Chromium in toys*
- *Arsenic in Urine*

Foodomics

Bacterial Proteotyping using Machine Learning defined peptide signatures and validation on a Q Exactive HF-X coupled to Capillary flow liquid chromatography

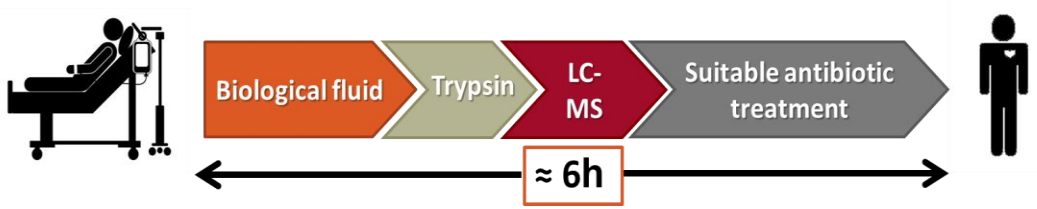
Introduction

According to the World Health Organization, bacterial infections cause millions of deaths each year. This is mainly due to **resistant** bacterial **infections** and to **time consuming analyses** required for a diagnosis.

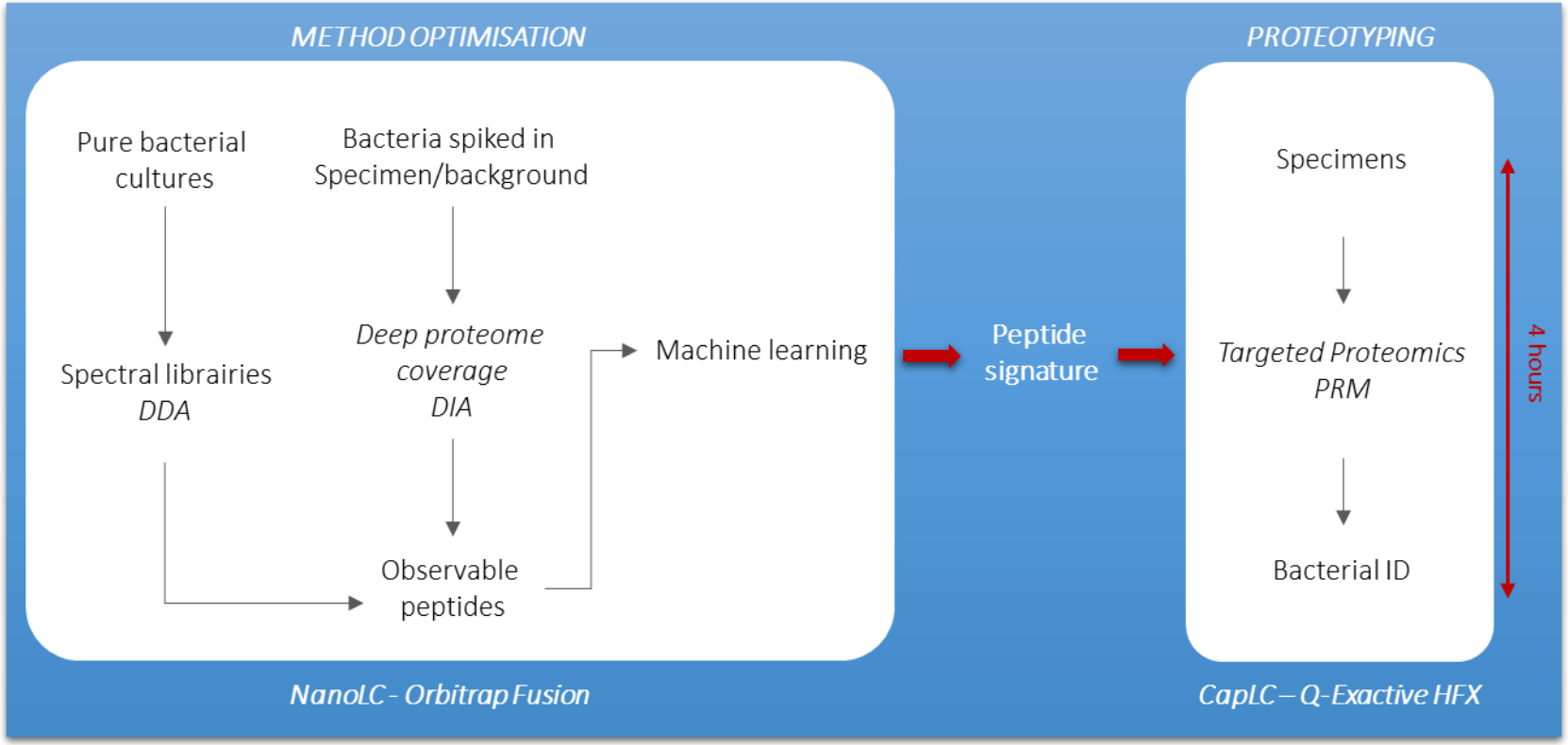


Over the five past years, MALDI-TOF mass spectrometry was implemented in most of the clinical laboratories However, this technique has several drawbacks: tests have to be done **on pure bacteria colonies** which are obtained after a lengthy protocol involving bacterial culture, moreover it **lacks specificity** for some bacterial species and is **non-quantitative.**

Our project aims to replace MALDI-TOF MS in clinical diagnosis by LC-MSMS approaches which would not require **any bacterial culture** because of it **high sensitivity**. This method could be applied to any biological sample susceptible to be infected by microorganisms and is quantitative.



Workflow

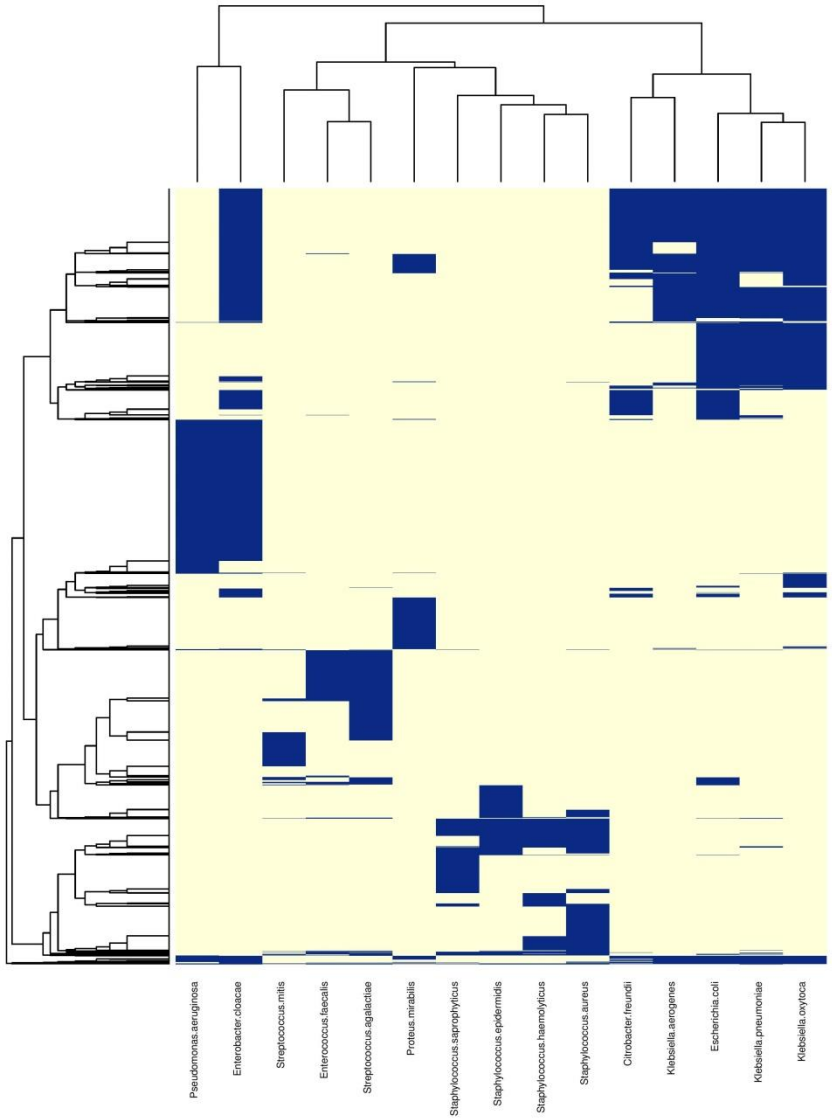


A **Peptide Signature** containing a short list of peptides able to distinguish between various species in sample is defined using:

- A deep proteome coverage of simulated infected samples by **DIA analysis**
- The use of **Machine Learning** algorithm

The Peptide Signature can be **monitored in targeted mode** on various types of instruments (MRM on triple quadrupoles, PRM on Q Exactive Orbitrap MS…) for **proteotyping** of thousands of samples.

Bacterial Proteomes Redundancy



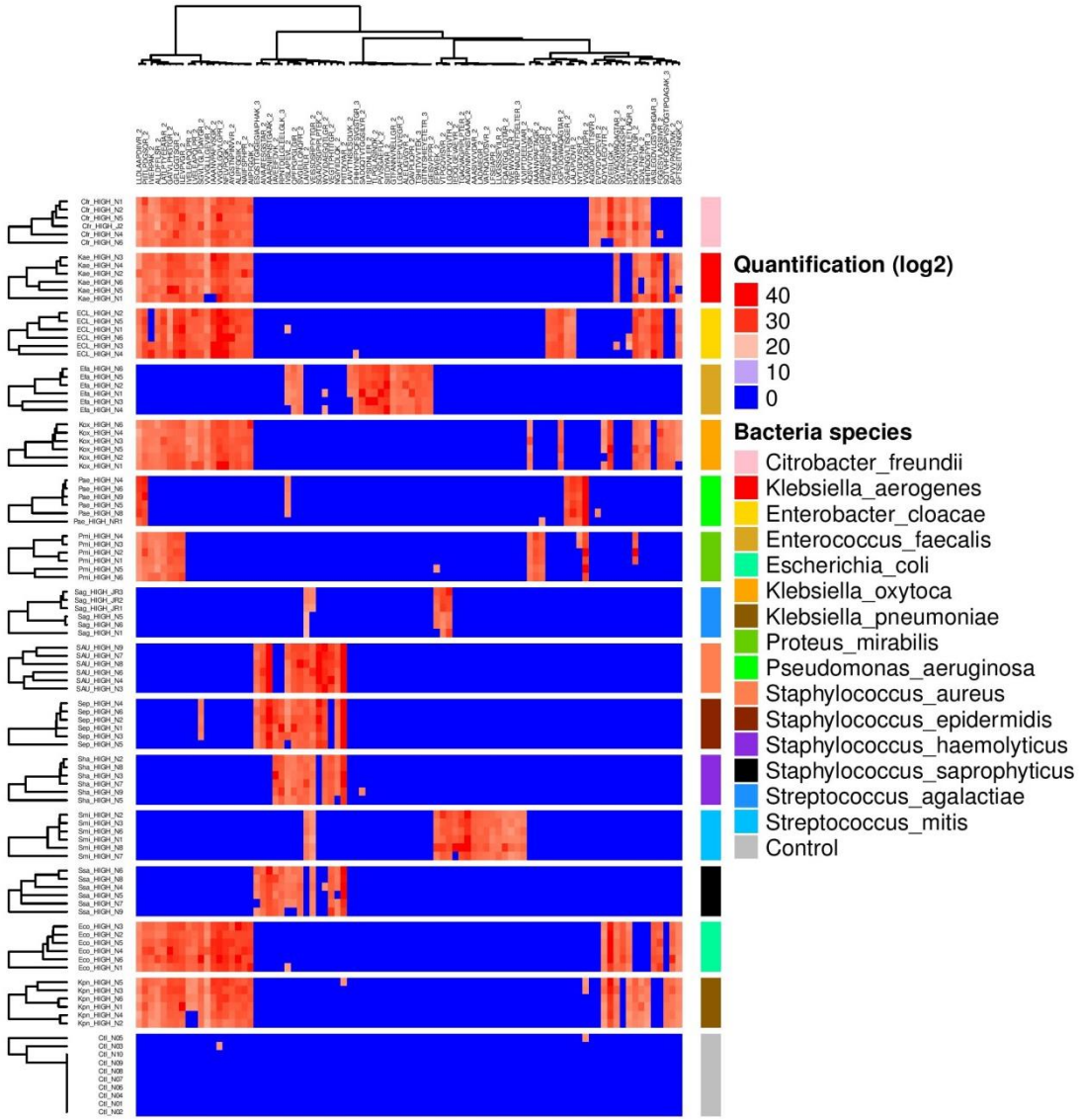
Application of the method to 15 bacteria most frequently found in UTIs

Generation of spectral libraries from single cultures for DIA**:**
10,000 to 20,000 peptides for each of the 15 bacteria

There is a **very high sequence redundancy** between the bacterial species, making it impossible to simply select unique peptides for each of them.

Heatmap of peptide identifications of each bacteria species (blue: detected, white: not detected). Bacteria and peptides are clustered using a hierarchical method.

Peptide Signature



Multiple replicates of each **bacterial species spiked in healthy urine** and analyzed by **Data Independent Acquisition mode**.

From these data, a Machine Learning algorithm (BayesNet) was used to generate a 87 peptides signature.

Although most of the peptides are shared by several species, **each species has a unique combination of peptides**.

Monitoring the whole signature in targeted proteomics allows a **proteotyping in less than 4 hours without bacterial culture**.

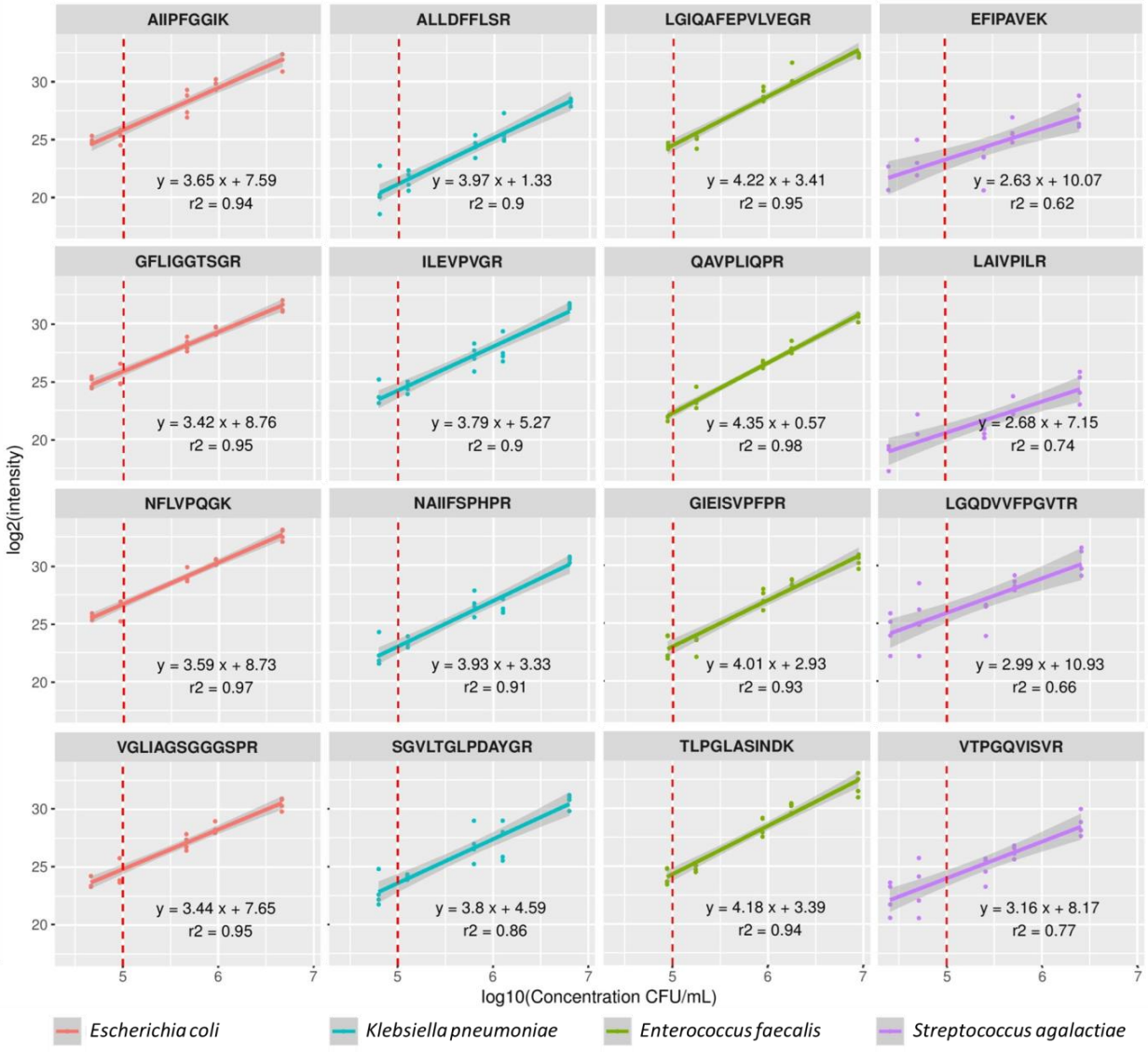
Heatmap of the Peptide Signature of 15 bacteria of UTIs showing the signal obtained for six spiked-in replicates in healthy urine by DIA analysis on a nano-LC Thermo Scientific™ Orbitrap Fusion™ system.

Linearity and Quantification

4 species were spiked at 5 different concentrations in healthy urine (ranging form 2^{e}4 to 8^{e}6 CFU/mL).

The Peptide Signature was monitored in **PRM (30 min LC gradient)** on a **CapLC-Q Exactive HF-X Orbitrap mass spectrometer.**
Very good linearities (R^2= 0.87 on average) were obtained for several peptides allowing their use for an **accurate quantification of the species in the sample**.

Peptide signal intensities after monitoring of the peptide signature in PRM mode on a CapLC-Q Exactive HF-X Orbitrap mass spectrometer of 5 concentrations of spiked-in bacteria in urine. The dotted red line represent the threshold usually used by clinical labs to consider an infection requiring antibiotherapy (1^{e}5 CFU/mL).



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Reproducibility over Biological Samples

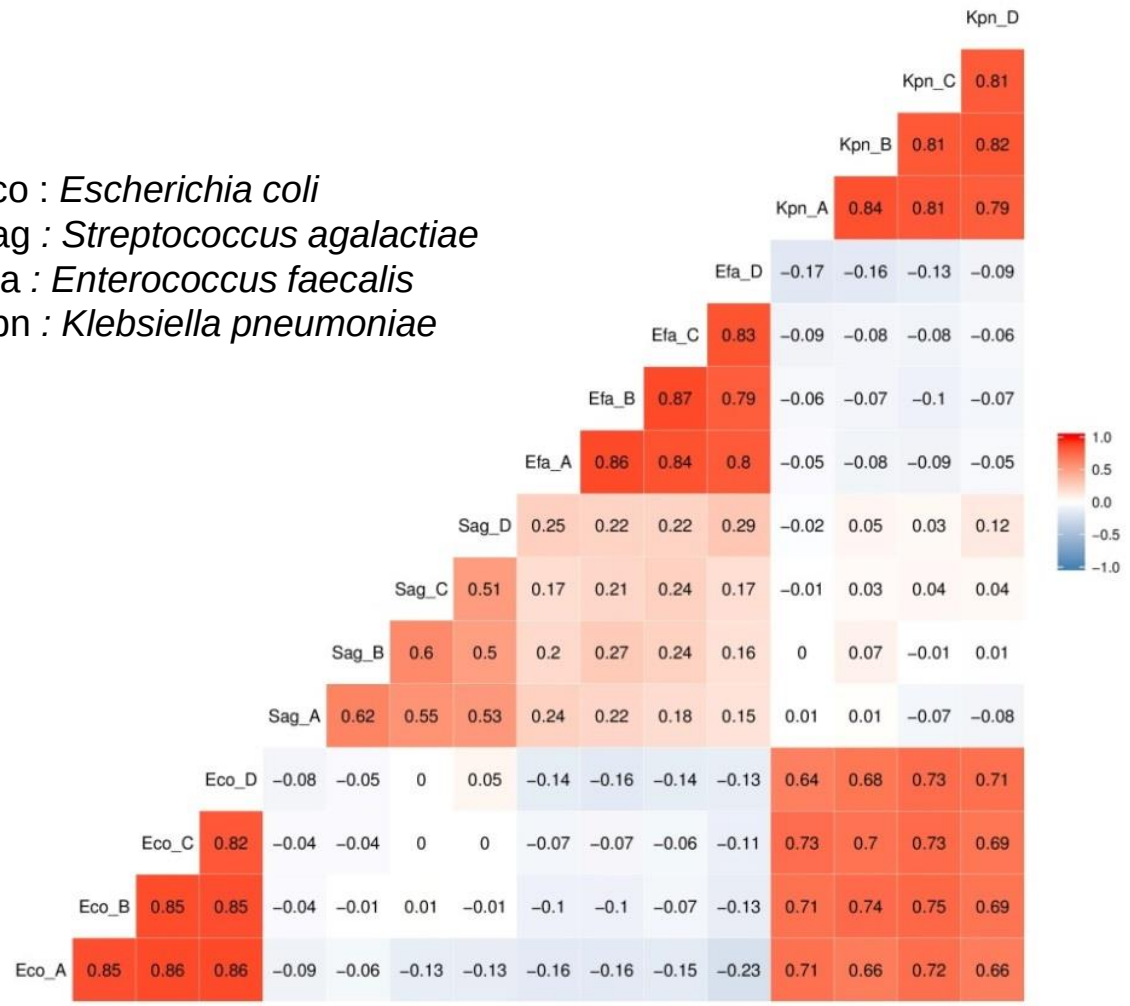
4 bacterial species were **spiked in 4 different healthy urine samples** at various concentrations. The Peptide Signature was monitored by **PRM on a CapLC-Q Exactive HF-X Orbitrap mass spectrometer.**

High Pearson correlation factors between replicates can be observed (0.76 on average) suggesting again the possibility of quantification.

A correlation is also found between *Escherischia coli* and *Klebsiella pneumoniae* because of their high redundancy. However, four peptides of the signature can be used to distinguish between the two.

Pearson correlation factors obtained after monitoring of the Peptide Signature of four replicates of four different bacterial spiked and at various concentrations.

Eco : *Escherichia coli*
Sag *: Streptococcus agalactiae*
Efa *: Enterococcus faecalis*
Kpn *: Klebsiella pneumoniae*



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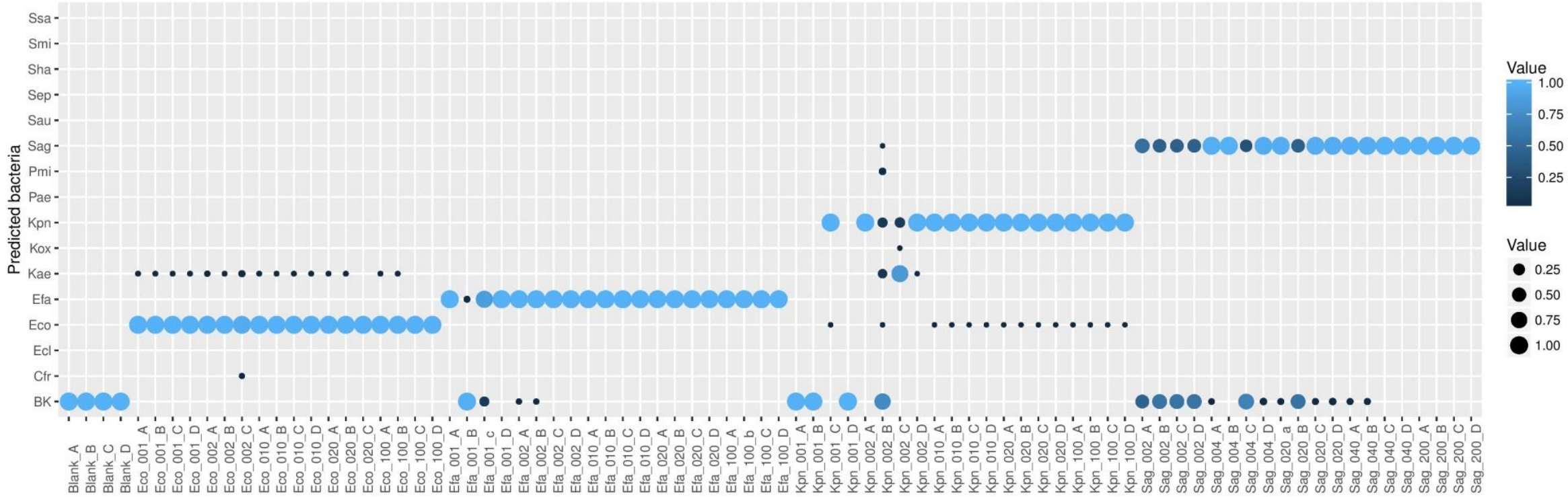
Bacteria Identification



Classification of spiked-in samples after monitoring of the Peptide Signature. Size and color of the dots show the probability of the prediction.

Four bacterial species were **spiked at 5 different concentration in healthy urine samples.** Signature was monitored by **PRM on a Q Exactive HF-X Orbitrap mass spectrometer.**

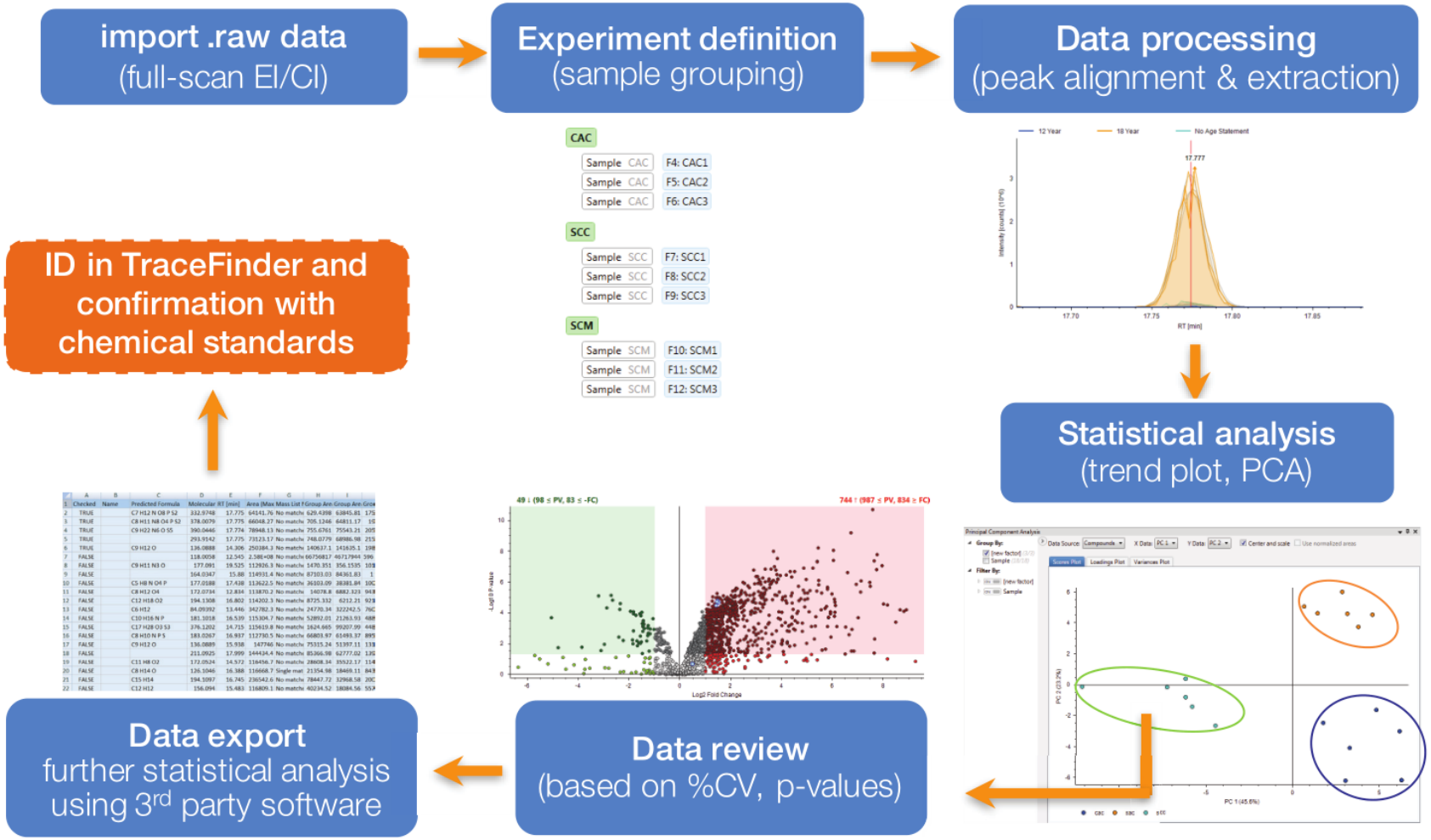
In 86% of cases, the bacteria infecting the sample was correctly predicted by the algorithm. For most of the others, the sample was reported as ‘blank’ because of very low spiked-in concentration (below the $1^{e}5$ CFU/mL threshold).

Conclusion

- We have developed a new strategy for bacterial proteotyping using LC-MS/MS able to identify the bacterial species infecting the sample in **less than 4 hours without bacterial culture.**
- We have successfully applied it on the 15 bacteria representing more than 90% of UTIs.
- In the future, this strategy could be extended to **any types of biological samples** in health, food or environment fields.
- Finally, this method could also be use for the detection of bacteria having **specific resistance or virulence**.

GC Orbitrap MS workflow for pathogenic microorganisms screening

- Application of GC Orbitrap mass spectrometry for untargeted metabolomics of pathogenic microorganisms



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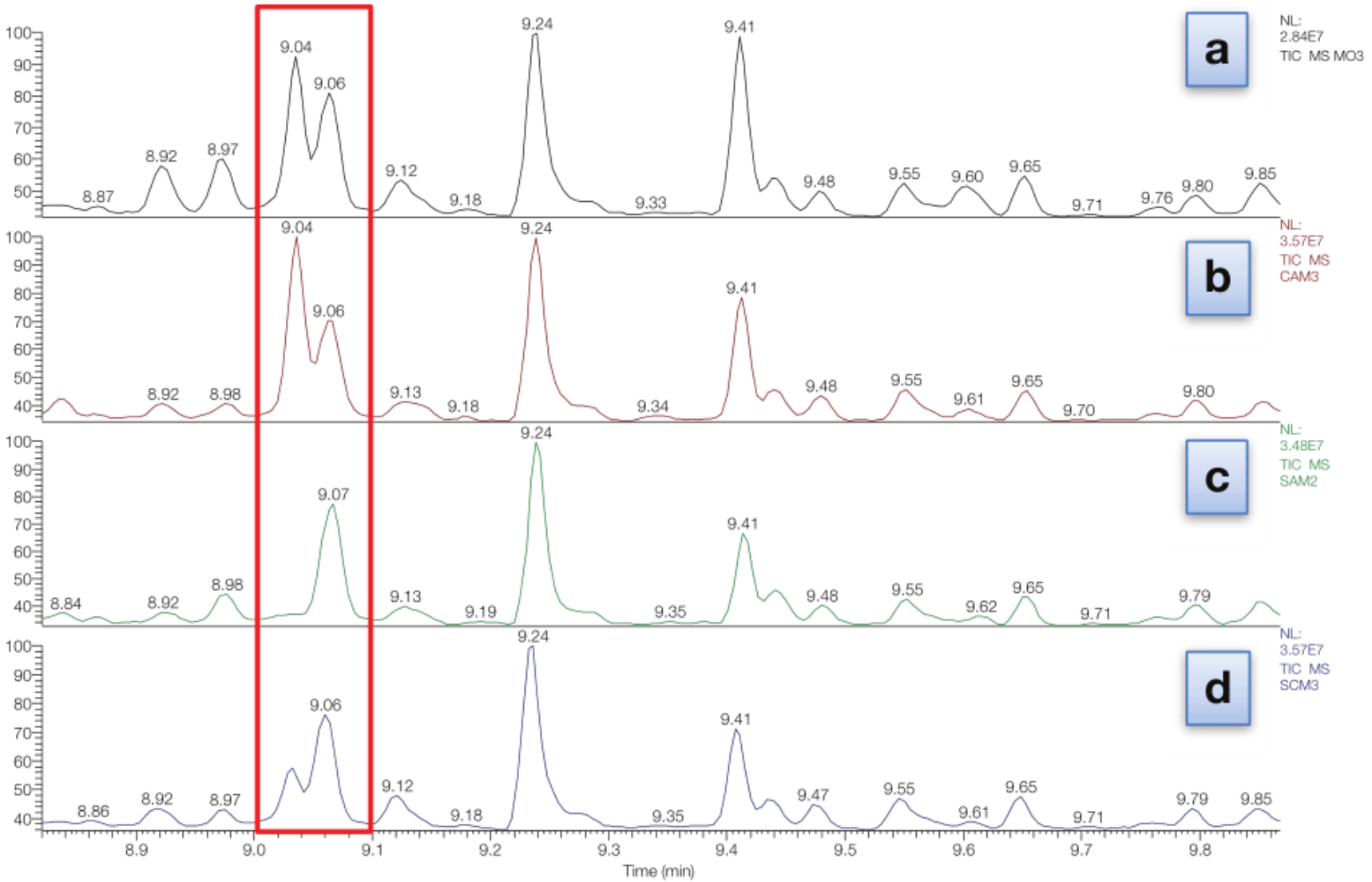
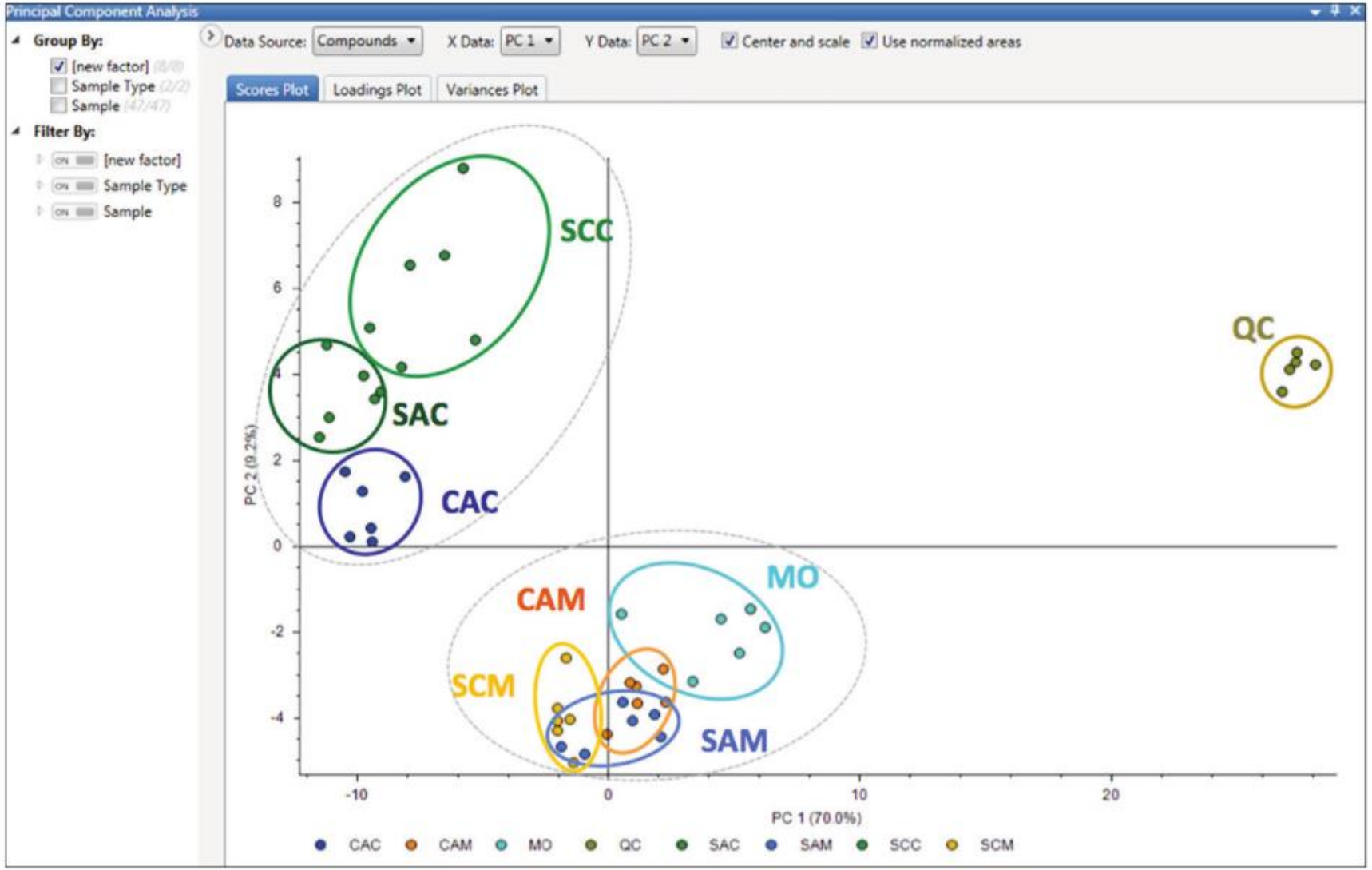
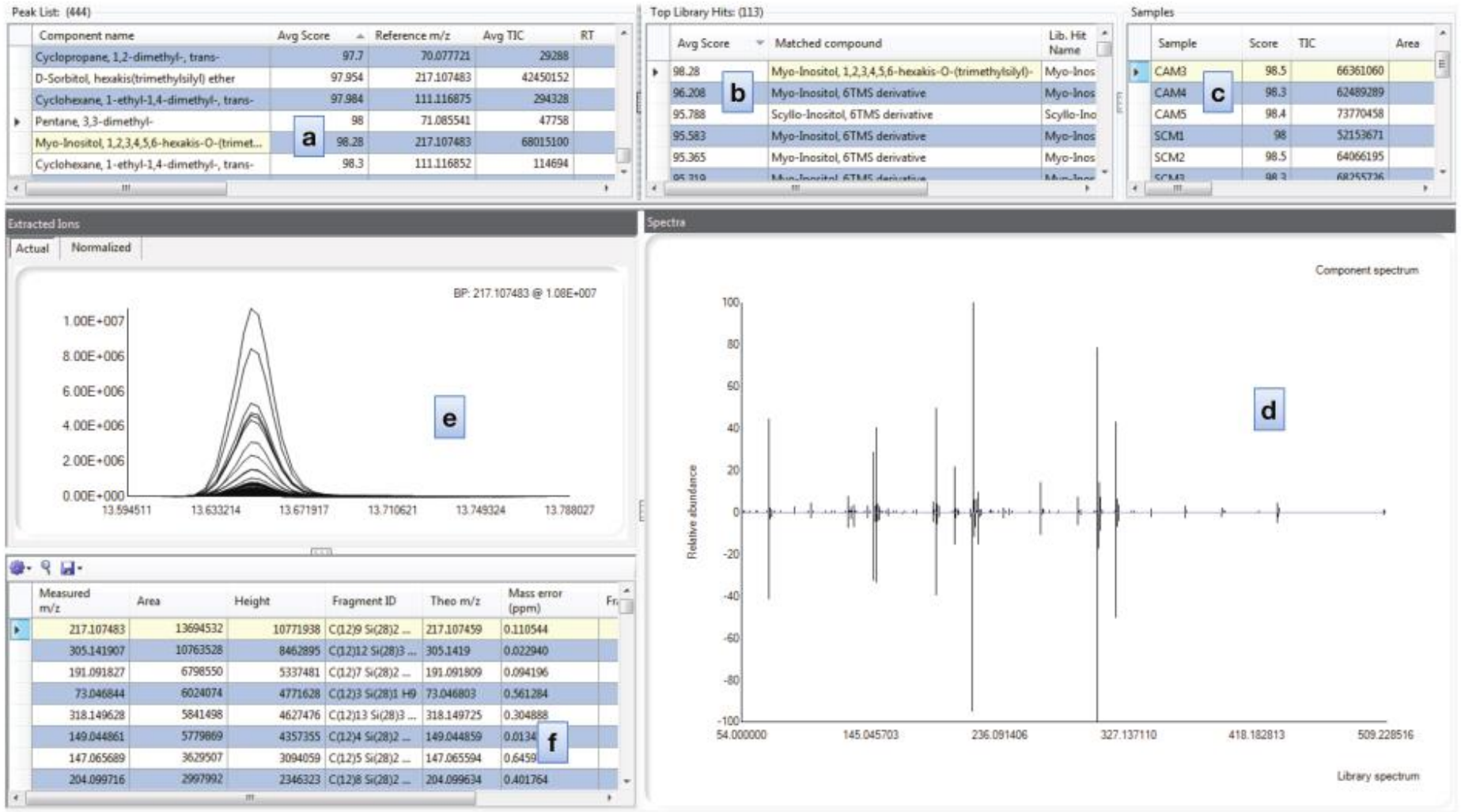


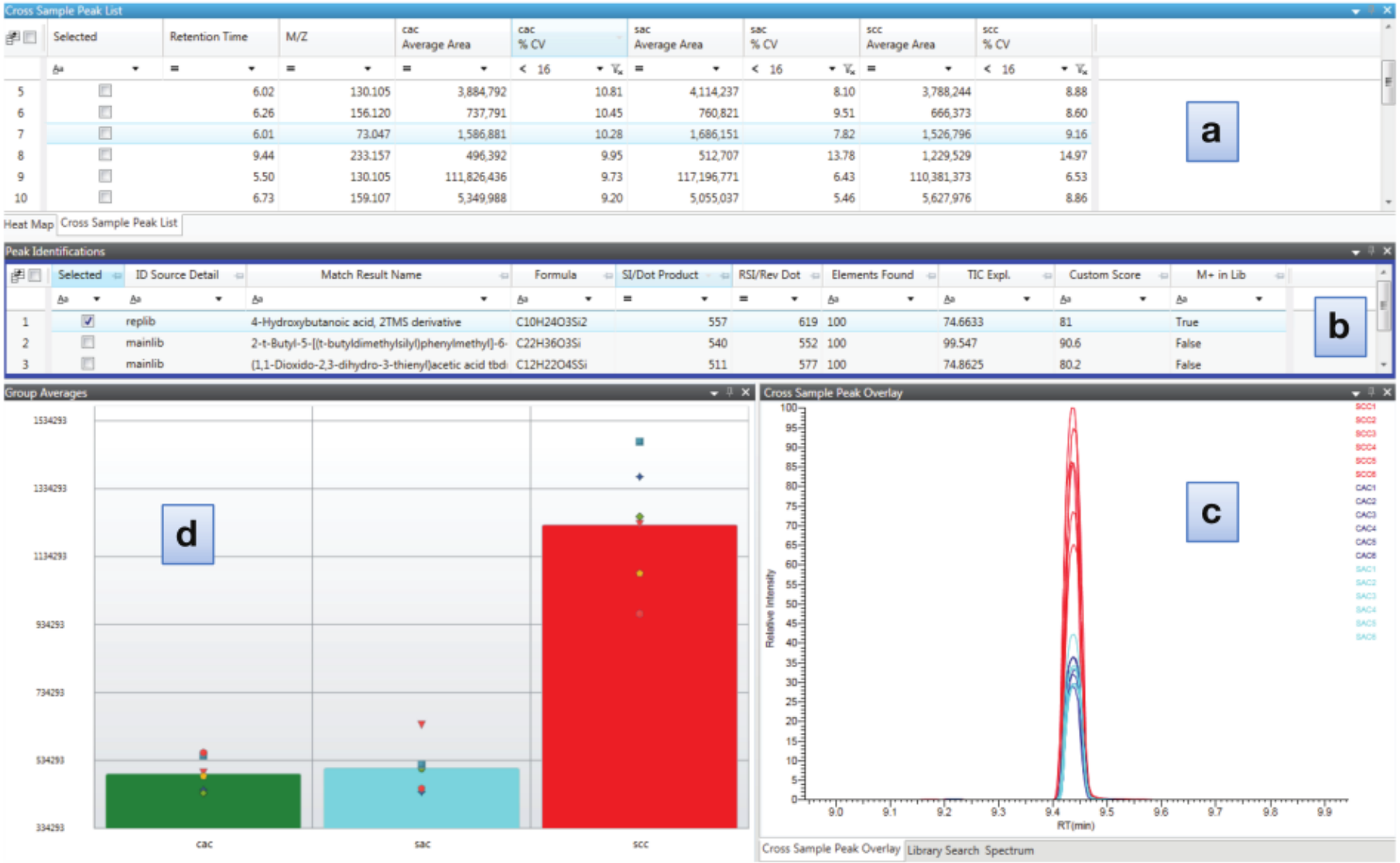
TIC chromatograms (EI, full-scan data) of media only (a), C. albicans media (b),S. aureus (c) and co-culture C. albicans and S. aureus (d). Highlighted is peak at RT = 9.04 min later identifi ed as glycine shows depleted levels in S. aureus media as compared to the other samples.



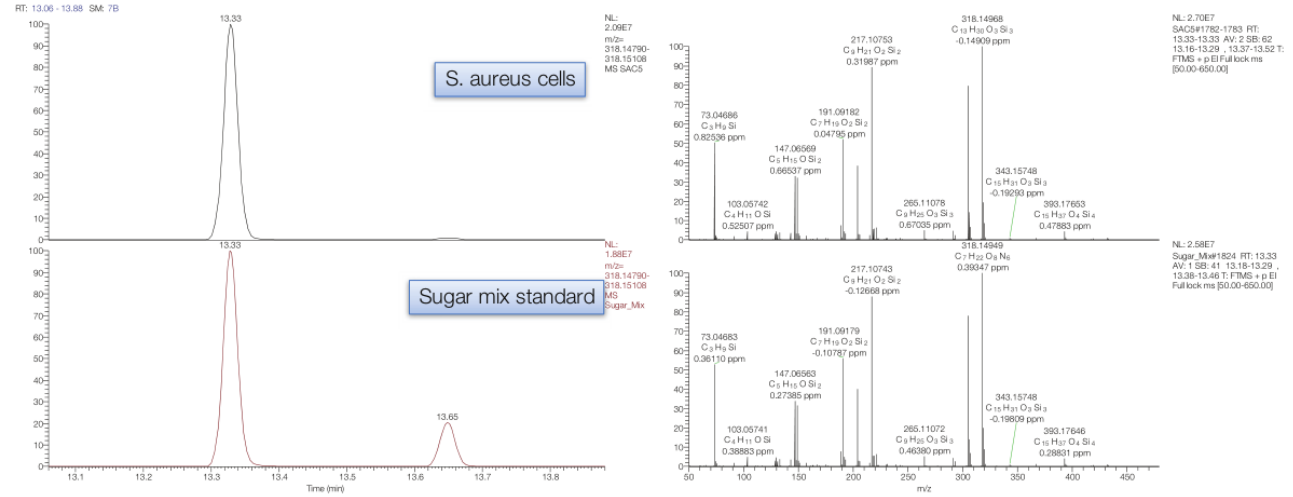
Principal component analysis (PCA) clearly shows a distinct separation between the media samples and the cell samples. Media only (MO), S. aureus media (SAM), C. albicans media (CAM), and media used for the co-culture of the two species (SCM) form distinct clusters. Intracellular metabolites extracted from S. aureus cells (SAC), C. albicans cells (CAC) and co-culture biofi lm (SCC) also form separate groups.

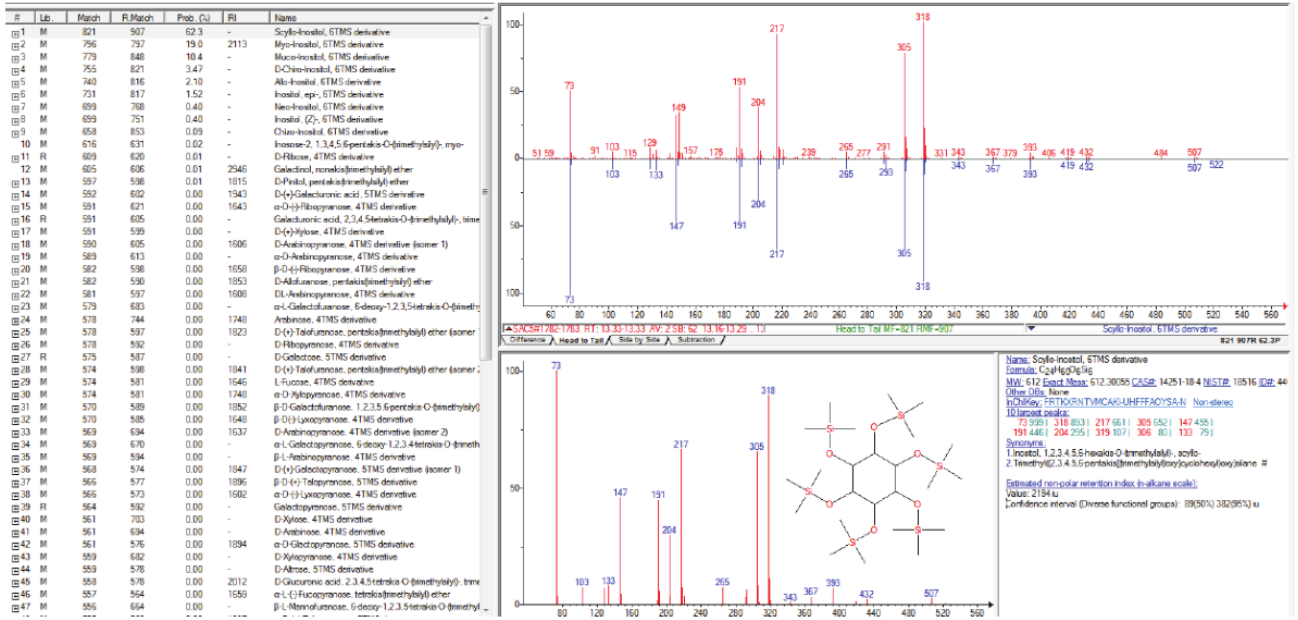


TraceFinder software peak deconvolution browser showing myo-inositol identification (a) based on a total (average) score (b) across the retention time aligned media samples (c). NIST spectral match (d), deconvoluted spectrum (e) as well as a list of the measured ions with their corresponding mass errors calculated taking into account the theoretical chemical elemental composition (f) are shown.



TraceFinder software browser showing cross sample retention time aligned peaks (a), peak identifi cation from the NIST search and HRF score (b), cross sample peak overlay (c) and peak intensity trend across media samples analyzed for selected compounds (in this case 4-hydroxybutanoic). Control sample is media only, C. albicans (CAM), S. aureus (SAM) and media used for co-culture of C. albicans and S. aureus (SCM).





Confirmation of scyllo-Inositol 6TMS derivative in S. aureus cell extracts, using a mixture of pure sugar standards. Library (NIST search index), retention time, spectral fidelity and sub ppm mass accuracy of measured fragment ions were used to confirm this compound.

Conclusion

- The results from these experiments suggest that most interactions between Candida albicans and Staphylococcus aureus are related to the synthesis and utilization of sugars as the main carbon source, in particular to the sedoheptulose-7-phosphate metabolism.
- Compound Discoverer and TraceFinder automate data processing, streamlining and simplifying the detection and confident identification of statistically significant metabolites.
- Importantly, the metabolomics workflow described here facilitates timely and confident data acquisition, data processing and interpretation of the results.
- The results obtained from these experiments demonstrate that the Q Exactive GC system is a powerful analytical tool that can be used to understand metabolic changes in complex bacterial interactions offering unprecedented insights into the pathogen-pathogen interactions at the small molecule level.
- Taken together, the Q Exactive GC mass spectrometer is a unique analytical tool able to detect a large number of metabolites with a simple setup and full-scan high resolution experiments.

THANK YOU!

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