UNIVERSITY OF CHEMISTRY AND TECHNOLOGY, PRAGUE
Faculty of Food and Biochemical Technology
Department of Food Analysis and Nutrition

Mass spectrometry based strategies for food authentication

Vít Kosek, Jana Hajšlová

EUCHINASAFE
中欧食品安全

Contents

- Reasons for food authentication
- Strategies for food authentication
- Analytical techniques
- Fingerprinting strategy

Reasons for food fraud

Deliberate fraudulent practice for financial profit
(adulteration / substitution – premium products/large quantities)

- Increasing price of some commodities (durum wheat)
- Insufficient supply (special teas, olive oil, saffron)
- Quality of the resources (bad harvest, variable composition)
- Restriction of tariffs (*e.g.* Basmati rice)
- Sustainability (illegal unregulated fishing)

Concerns about authenticity

Minor problems:

- Bad production practice
- Inexperienced producer
- Inadequate control of basic resources
- Inadequate control of products

Major problems:

- Deliberate use of (cheap) resources inappropriate for the final product

Deliberate consumer deception

Reasons for authenticity control:

- Consumer protection (food quality and safety)
- Producer protection (protection from competition, which sells cheaper but adulterated products)

Authenticity control

Requirements for authenticity control:

- **Knowledge of processing technology, composition of the product and the ingredients**
- **Employment of advanced analytic techniques**
- **Experienced analyst**

Assessment of compliance:

- With legislation
- with information on the label

➲ ***Detection of compounds/attributes specific for adulterants***

➲ ***Decrease or change of compounds specific for ingredients of the product***

Constant development of new analytical strategies is necessary

Authentication

Food authentication is a process which aims on verification of information about constitution, processing and origin of the product as stated on the label.

Steps that can be taken to evaluate product authenticity?

- Check of the documents
- Sensoric analysis
- Analytical evaluation – complex chemical composition, aromatic profiles, typical compounds, physicochemical properties

Necessary requirement:

Accredited labs with standard analytical methods which can answer the question about product authenticity

Authentication

Food authentication is a pr… …cation of information about constitu… …n of the product as stated on the lab…

Steps that can be taken to e… …?

- Check of the documents
- Sensoric analysis
- Analytical evaluation – co… aromatic profiles, typical c… …n properties

In order to have standard methods, state-of the art methods are required

Necessary requirement:
Accredited labs with standard analytical methods which can answer the question about product authenticity

Basic authentication strategies

Every authentication strategy must be scientificaly sound. i.e. must be precise, reproducible to reliably decide whether the product is authentic.

Present:

- Analytical methods require statistical evaluation of the data

Future:

- Evaluation of big datasets and their analysis by MVA
- Integration of geographical information systems with climate information systems
- Metabolomic analysis to determine the precise information about origin of the product, geographical origin of ingredients and year of harvest.
- ….

Food composition

Natural components

Natural toxins

Antinutrition comps.

Primary sensorically active comp.

Antioxidants and other biologically active components

Nutrients

proteins
lipids
saccharides
minerals
vitamins

Fiber

DNA
RNA

Contaminants

Environmental contaminants

Pesticide / veterinary drug residues

Migrants from plastics

Toxic metals

Processing products

Additives

Biotechnology products

Food fraud discovery scheme

Authentic sample set S_n

Suspect sample X

Analytical strategies

Matrix of results S_n

S_{11}, S_{12} ...S_{1n}

S_{21}, S_{22},...S_{2m}

Analysis results for X

Model for similarity testing:

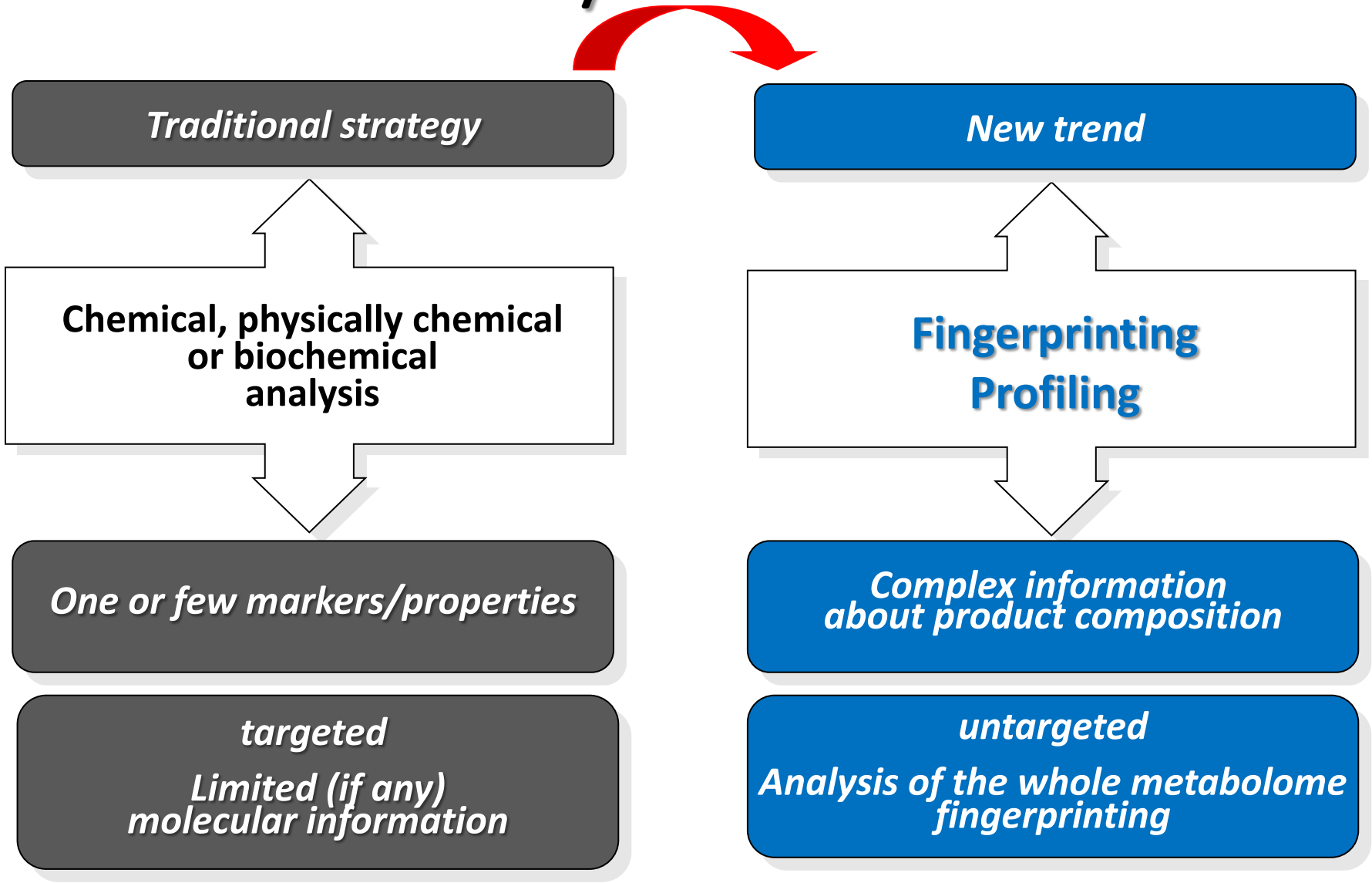
Is sample X compared to S_n typical or atypical ?

NON-COMPLIANT

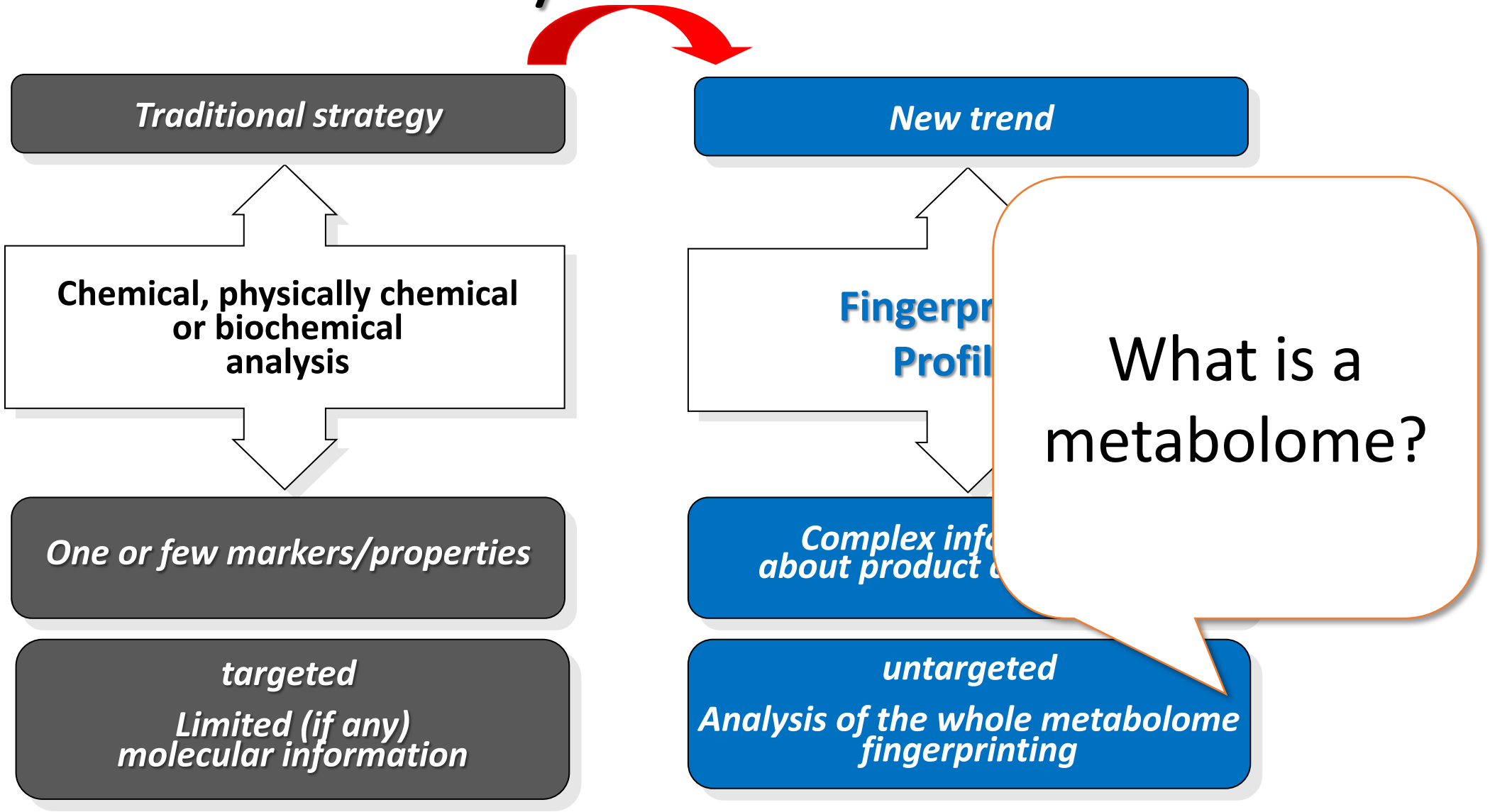
COMPLIANT

Importance level (α)

Food fraud discovery

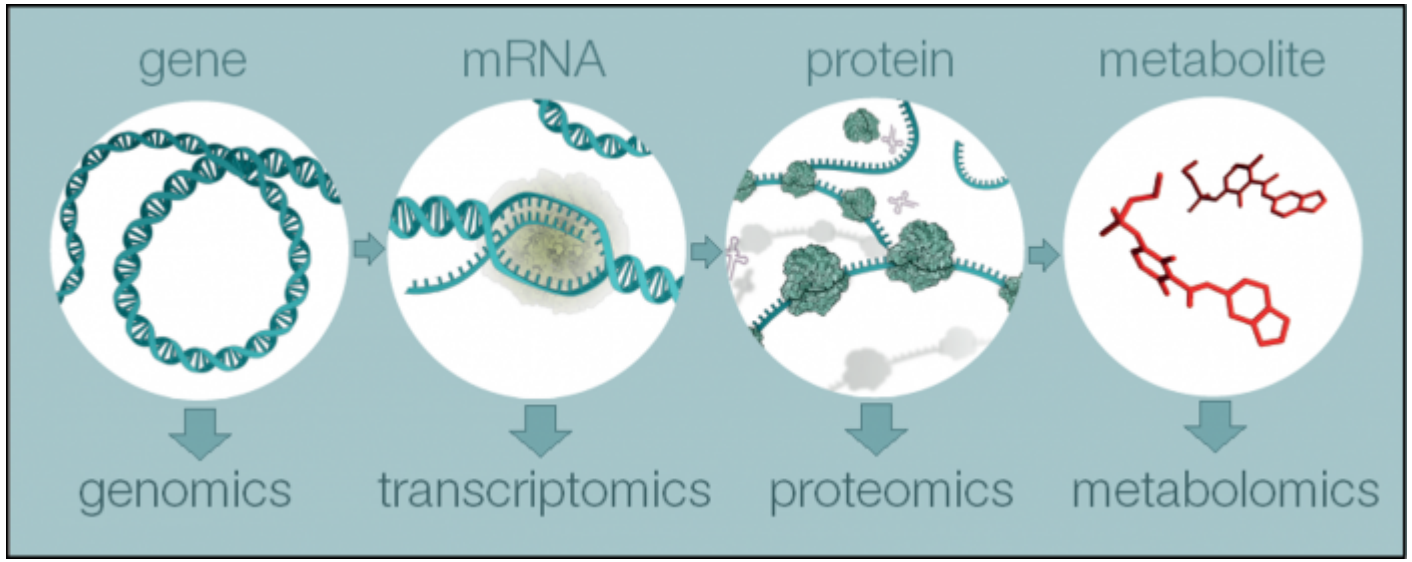


Food fraud discovery



Central dogma of systems biology

- **-omics:** Unbiased **large-scale analysis** of a set of molecules present in the biological system



https://www.ebi.ac.uk/training/online/sites/ebi.ac.uk.training.online/files/user/2760/images/Metabolomics/central_dogma_figure_1_.png

- **Metabolomics:** directly related to biological activity *i.e.* **phenotype** and **biological state**

Metabolome

- A set of small molecules inside an organism (matrix)

METABOLOME IS INHERENTLY VERY DYNAMIC

interaction both within and between biological systems, and with the

EXTERNAL ENVIRONMENT

climatic conditions ◆ soil ◆ pests ◆ agrochemicals

CULTIVAR

Metabolome

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CULTIVAR

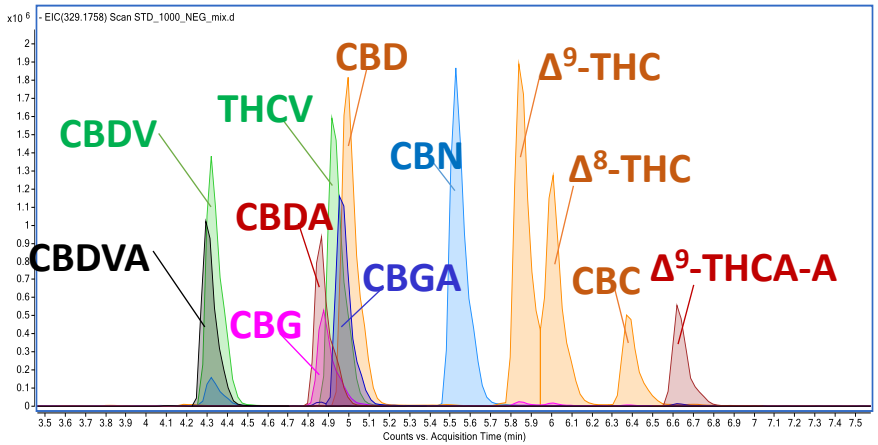
Foods contain metabolites from their constituents and many more molecules!

Technology ◆ storage ◆…

Targeted authentication strategies

Quantitation

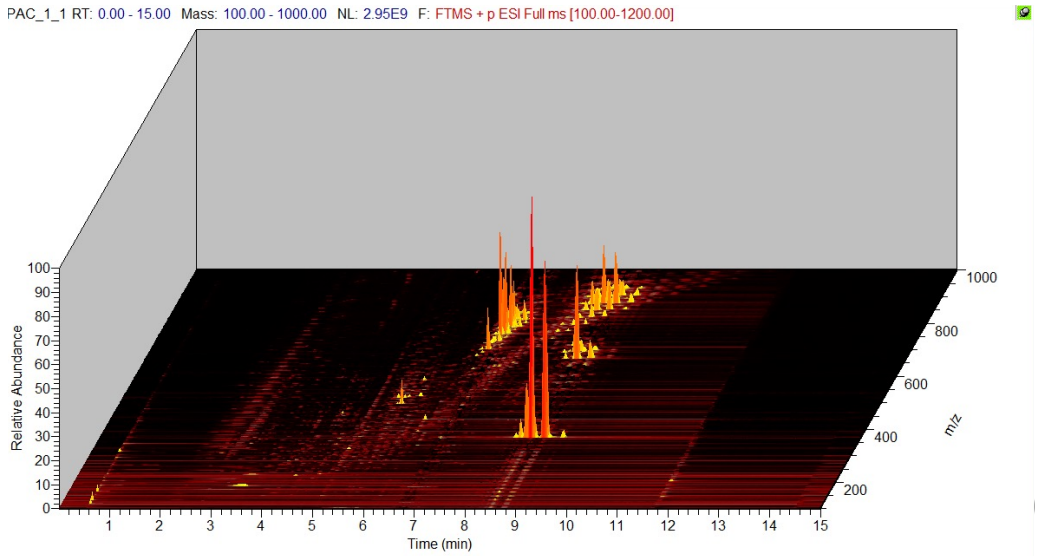
- Targeted
- Quantitative
- Coverage of small set of compounds
- Biased
- Hypothesis driven *e.g.* Cannabinoid metabolism for Cannabis cultivar authentication



Targeted authentication strategies

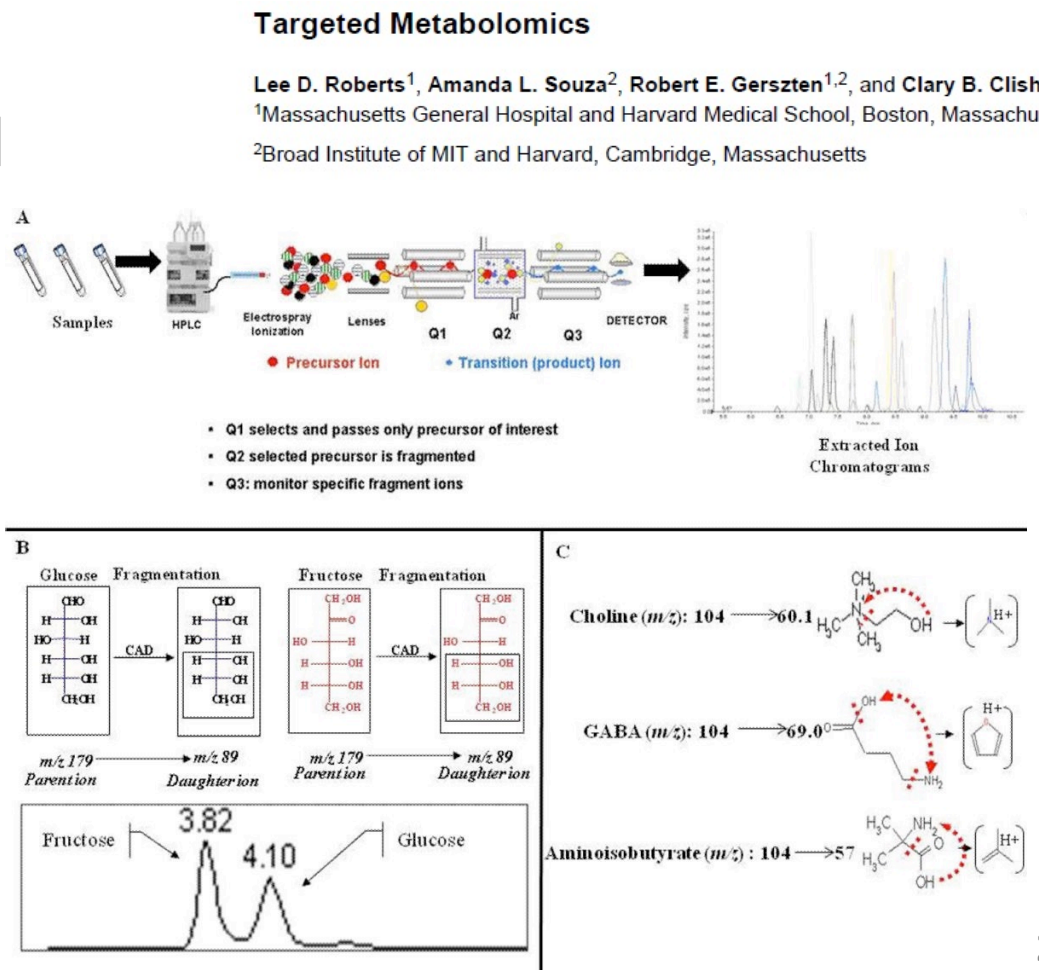
Profiling

- Targeted
- Non-quantitative
- Coverage of larger set of metabolites
- Biased – commonly a group of compounds related by structure or function
- Hypothesis driven *e.g.* phenolics for wine authentication



Requirements for targeted techniques

- Basically the same for usual targeted methods
- Specificity
- Full method validation usually required for quantitation
- High throughput



Untargeted strategies for authentication

Fingerprinting

- Non-targeted
- qualitative
- Coverage of large set of compounds
- Least biased
- Hypothesis generating

Requirements for untargeted analytical techniques

- Full spectral information
- Generic conditions
- High throughput (if necessary)
- Good interpretability of the signals is recommended

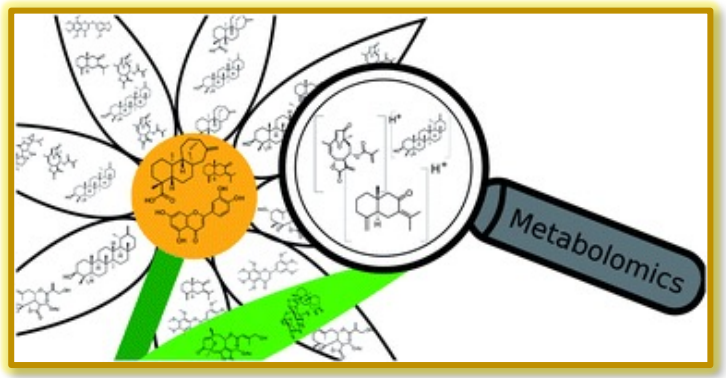
Molecular resolution

Instrumental platforms for fingerprinting

Ambient/DI-MS

SFC/LC/CE–MS

METABOLOME



ICP–MS

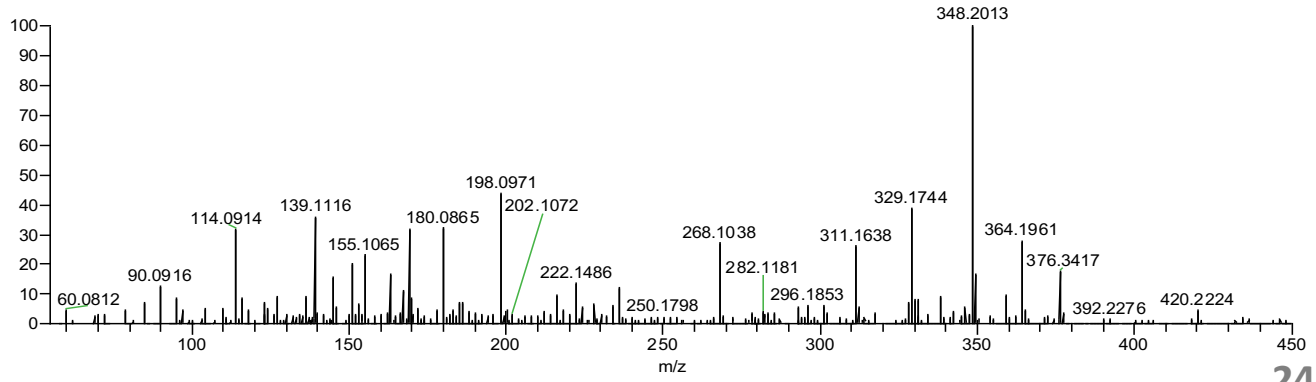
NMR

GC-MS
SPME-GC-MS

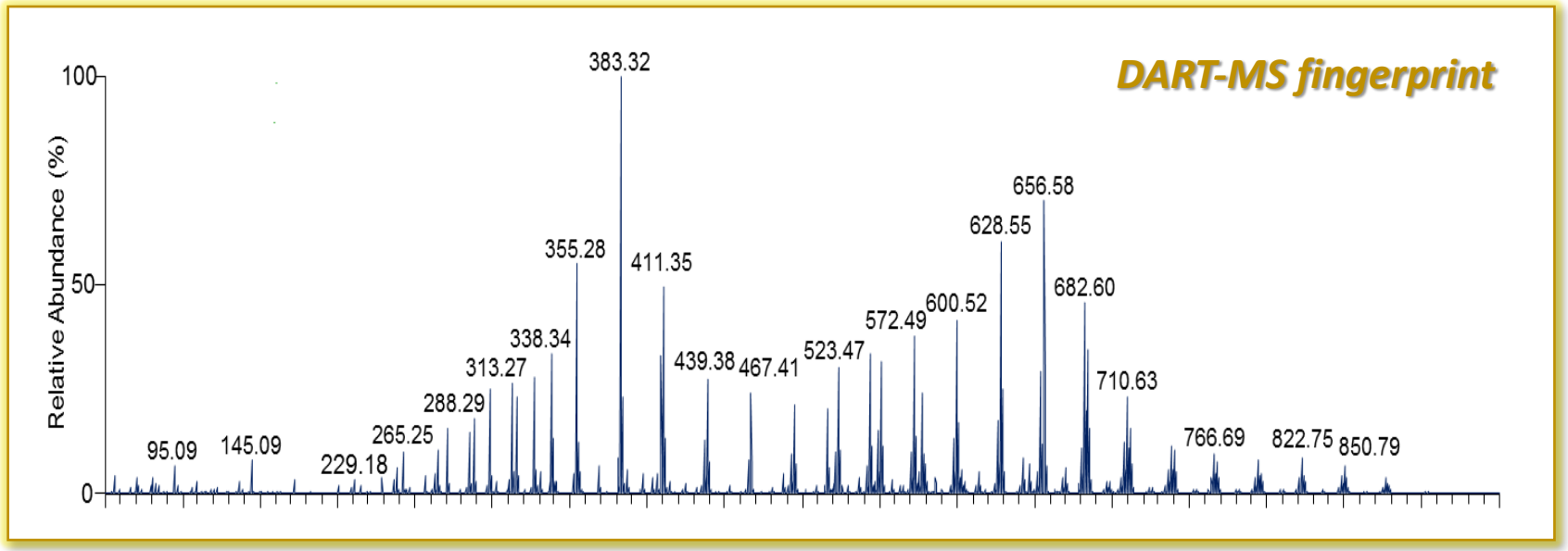
IR / Raman

Mass spectrometry

- Weighing molecules
- Molecules need to be ionised
- Ions can be manipulated inside electric or magnetic field
- Mass spectrum: *m*/*z* X intensity
- Destructive X very sensitive
- Specific



Mass spectrometric fingerprint (snapshot)



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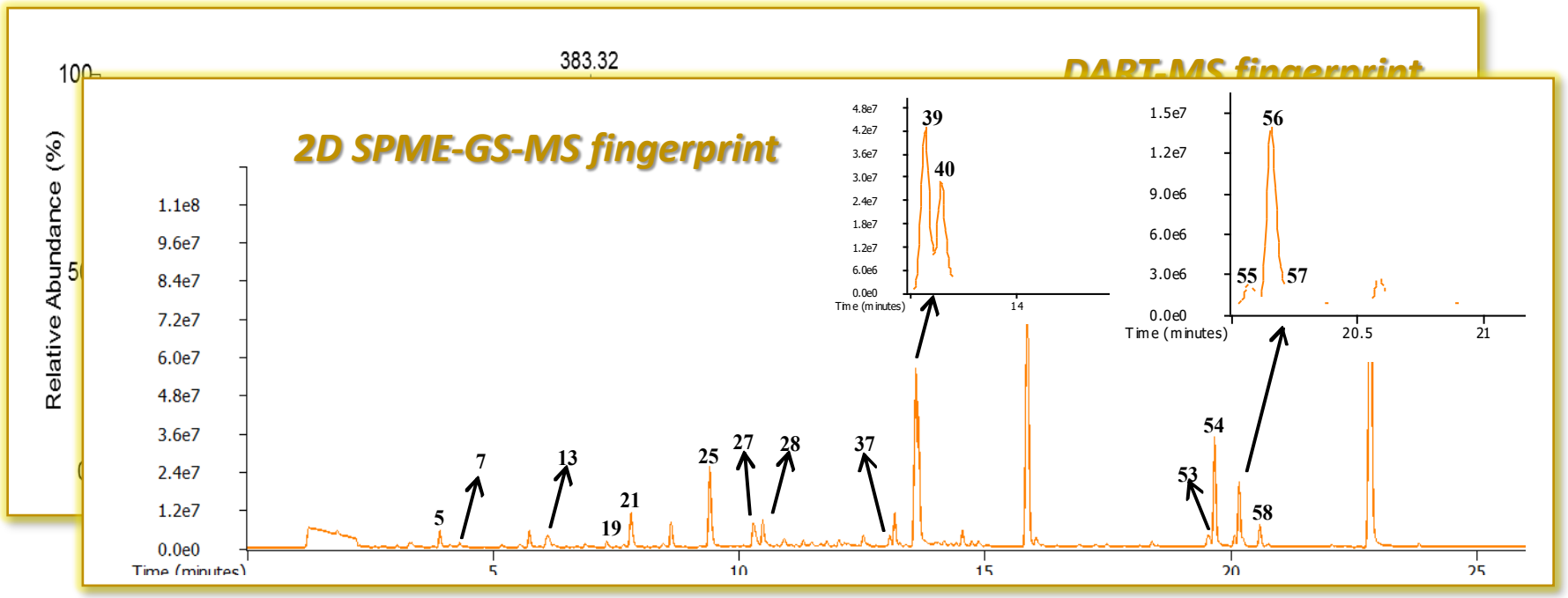

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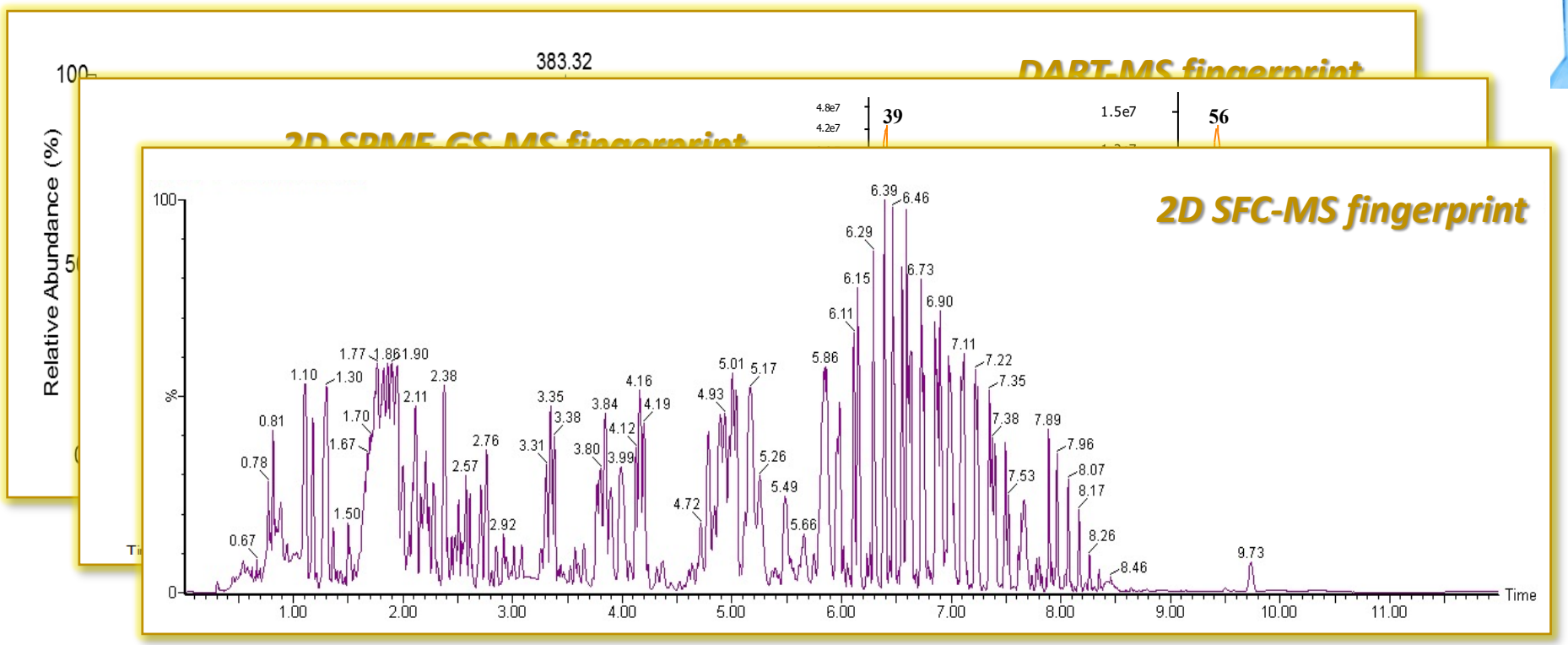

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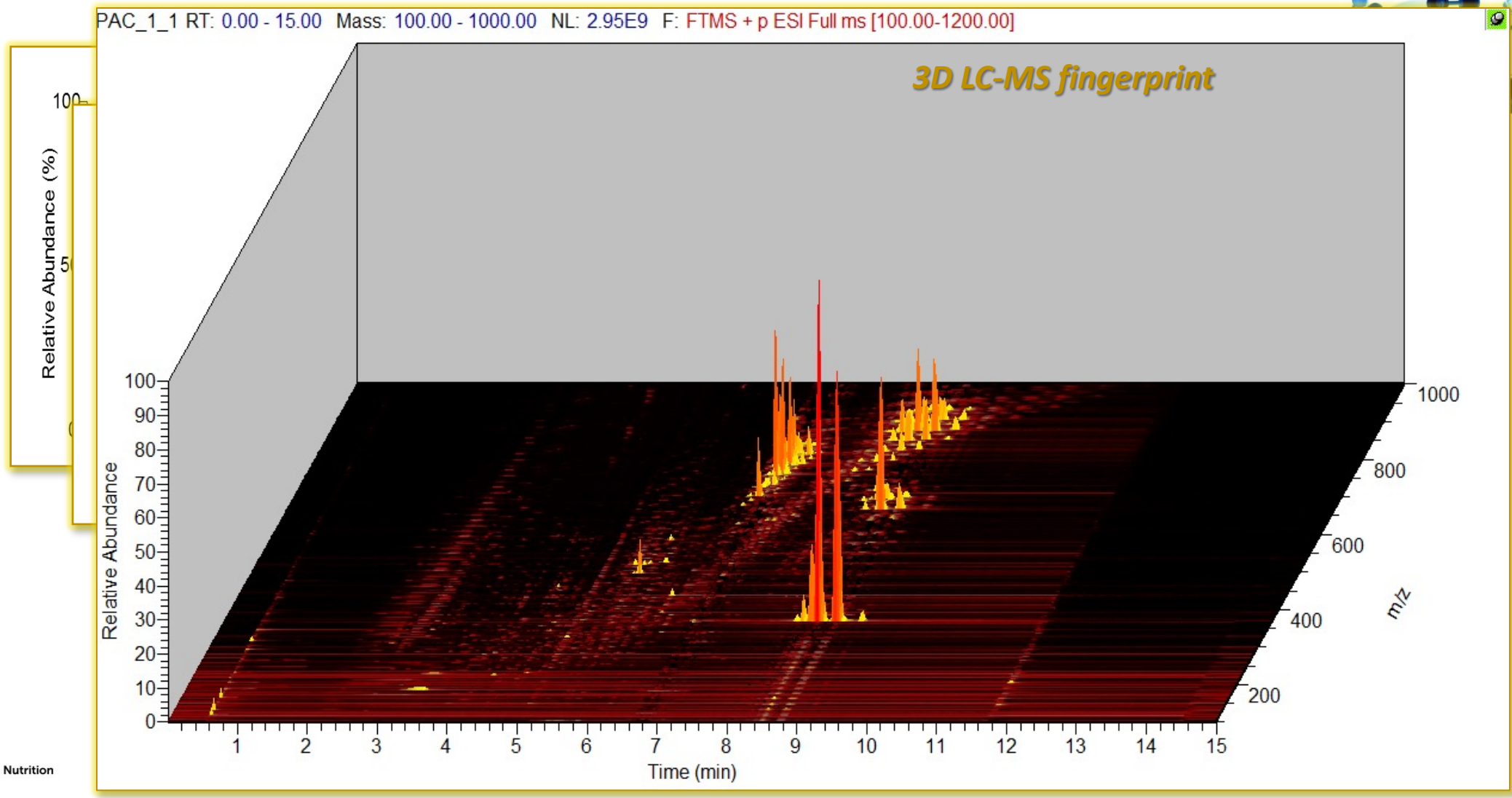
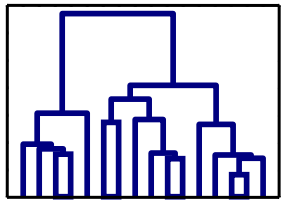
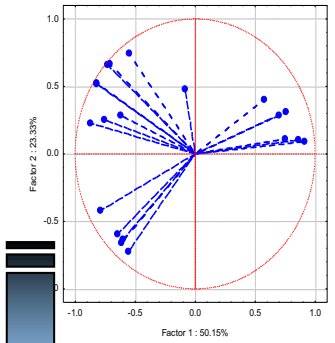


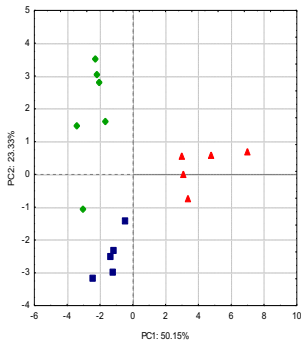
Basic food fraud discovery strategy

Samples

Data analysis



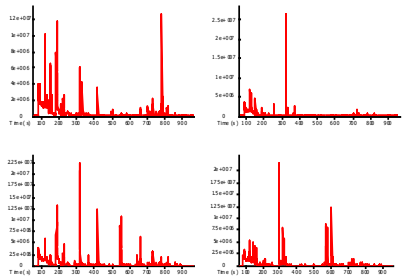




Compliance with:

- legislation
- Information on the label

Analysis



Raw data

No.	Group	Var1	Var2	Var3	Var4	Var5	Var6	Var7	Var8	Var9	...	Var33
1	EVOO	0.488	0.158	0.217	0.564	0.069	0.242	0.115	0.064	0.019	...	0.005
2	EVOO	0.496	0.166	0.222	0.580	0.070	0.249	0.057	0.033	0.020	...	0.013
3	EVOO	0.083	0.043	0.032	0.001	0.022	0.140	0.001	0.001	0.008	...	0.002
...	...	...	...	...	...	...	...	...	...	...	...	...
11	HO	0.866	0.045	0.736	0.029	0.026	0.025	0.000	0.004	0.010	...	0.011
12	HO	0.853	0.043	0.703	0.028	0.026	0.024	0.000	0.004	0.009	...	0.000
13	HO	0.724	0.027	1.000	0.016	0.043	0.049	0.000	0.003	0.007	...	0.014
...	...	...	...	...	...	...	...	...	...	...	...	...
21	OO	0.264	0.098	0.060	0.015	0.028	0.153	0.000	0.001	0.015	...	0.006
22	OO	0.273	0.097	0.061	0.015	0.029	0.154	0.000	0.001	0.015	...	0.005
23	OO	0.435	0.016	0.010	0.000	0.012	0.165	0.000	0.001	0.002	...	0.000
...	...	...	...	...	...	...	...	...	...	...	...	...
38	OPO	1.000	0.008	0.002	0.000	0.020	0.092	0.005	0.004	0.001	...	0.014
39	OPO	0.783	0.007	0.000	0.005	0.045	0.036	0.000	0.002	0.000	...	0.022
40	OPO	0.849	0.000	0.000	0.006	0.051	0.038	0.000	0.004	0.000	...	0.026

Data matrix

Fingerprinting workflow

- All the parts must be carefully planned
- Quality control must be maintaned during the whole process

- *Sample size*
- *Variability*
- *Confounding factors*
- *...*

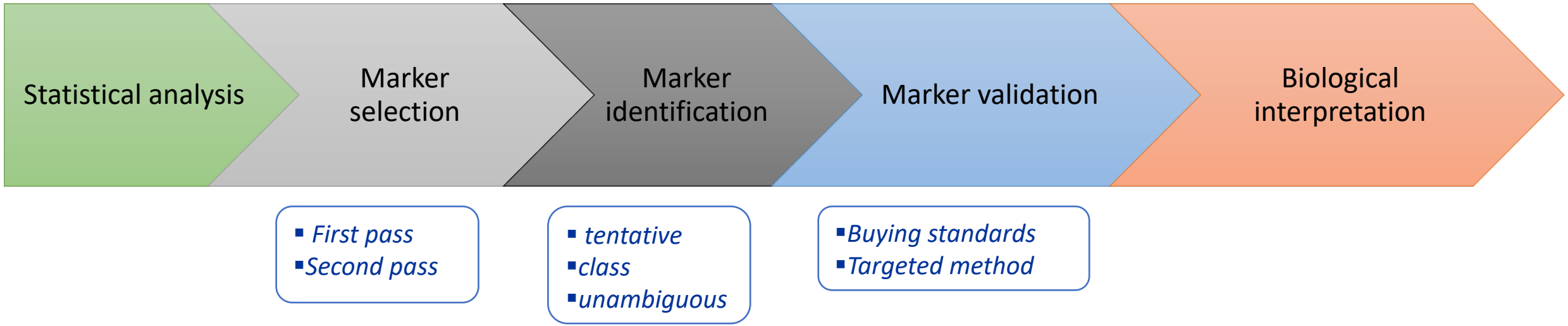
- *Normalisation*
- *Extraction*
- *Repeatability*
- *QC preparation*
- *...*

- *Normalisation*
- *Internal standards*
- *Batch effect*
- *Criteria for peaks selection*
- *Repeatability*
- *...*

- *Overfitting*
- *Models validation*
- *...*

Fingerprinting workflow

- Data processing, statistical analysis and interpretation is usually the most time consuming steps



Sample preparation

- Generally very simple -> reduction of error
- Generic methods are used -> increasing coverage
- Derivatization may be neded for certain analytes

Data acquisition

Requirements:

- Stable AND repeatable conditions throughout the measurement
- Quality control procedures

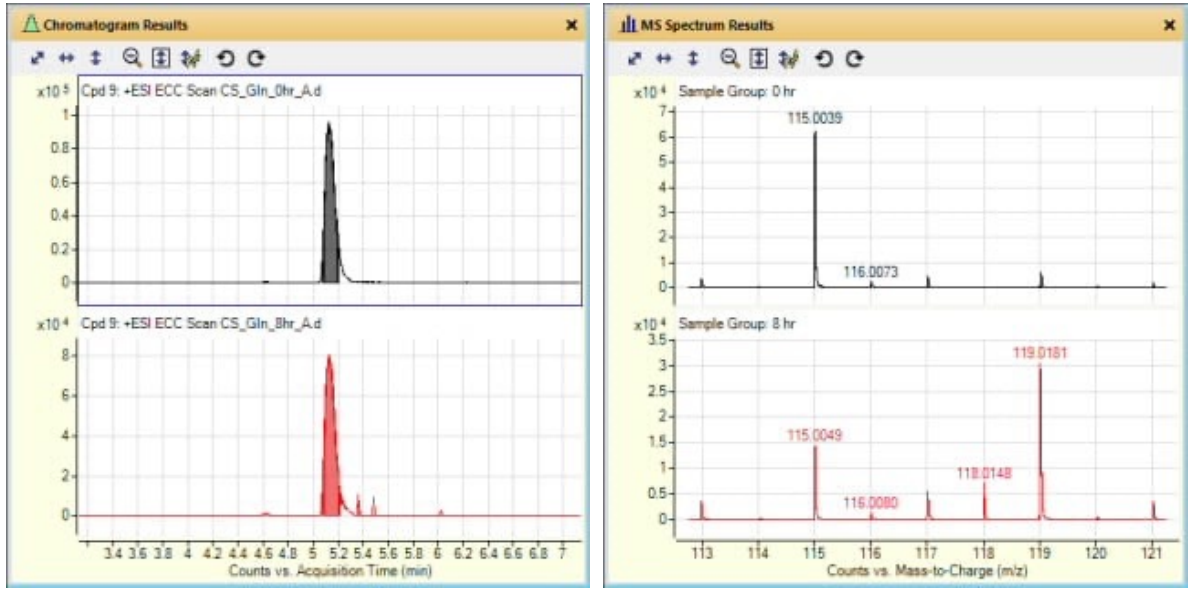
- Incorrectly acquired data -> useless results
- A compromise between data complexity, speed and limits of detection

Data processing

- One of the most crucial steps in the workflow

Bad data + good processing = bad results
Good data + bad processing = bad results
Good data + good processing = **good results**

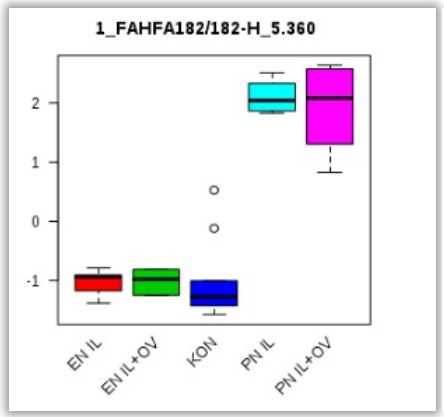
- Peak picking
- Peak alignment
- Data normalization
- Transformation
- Scaling

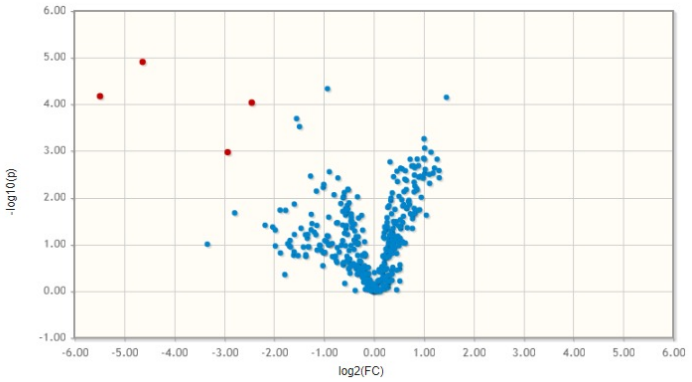


Statistical analysis

Univariate statistics

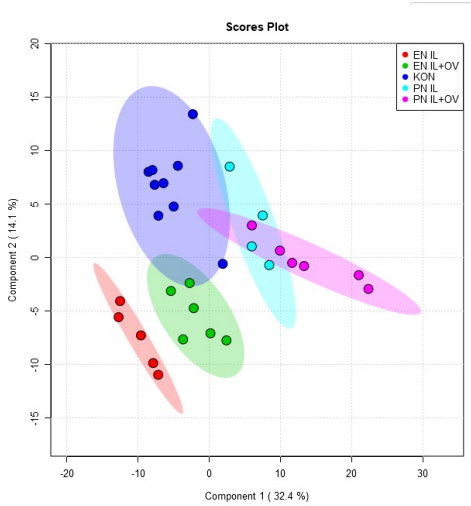
- More traditional approach
- Useful for individual variable evaluation and filtering

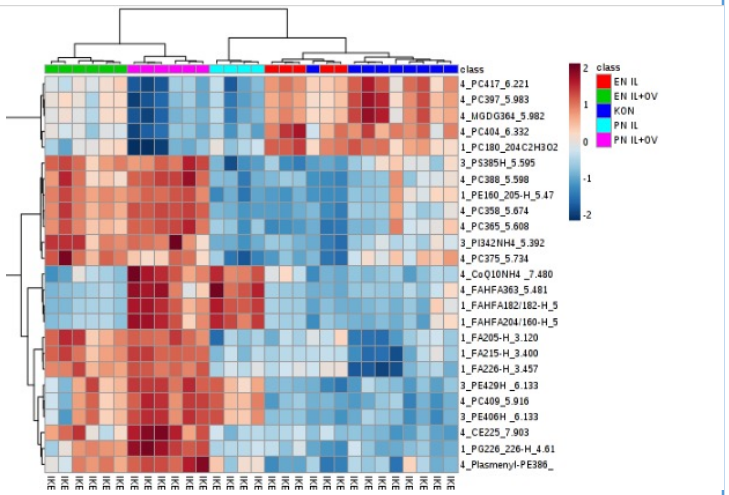




Multivariate statistics

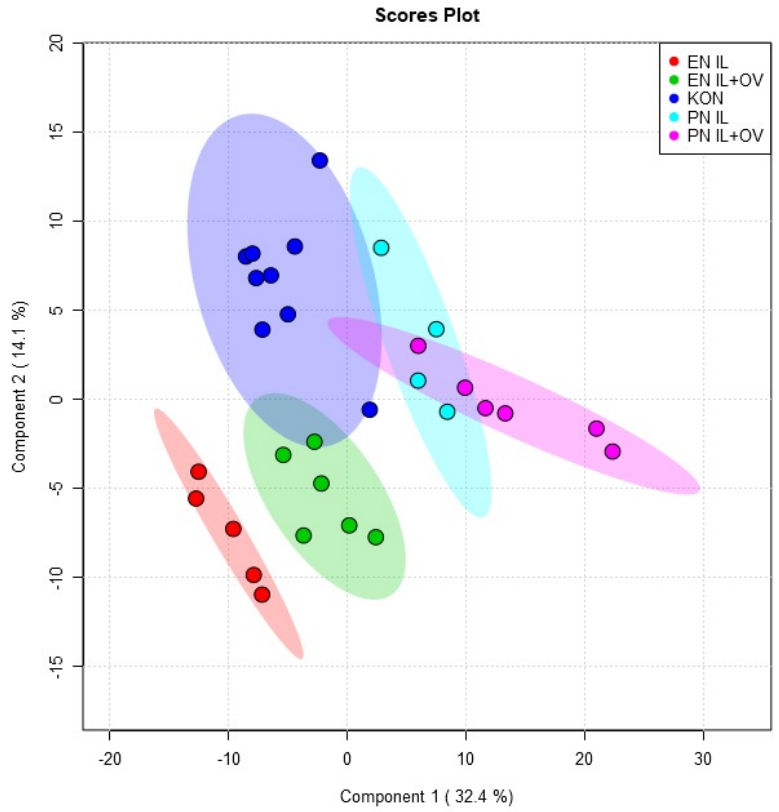
- Simplification of multidimensional data
- Useful for evaluation of patterns in the data



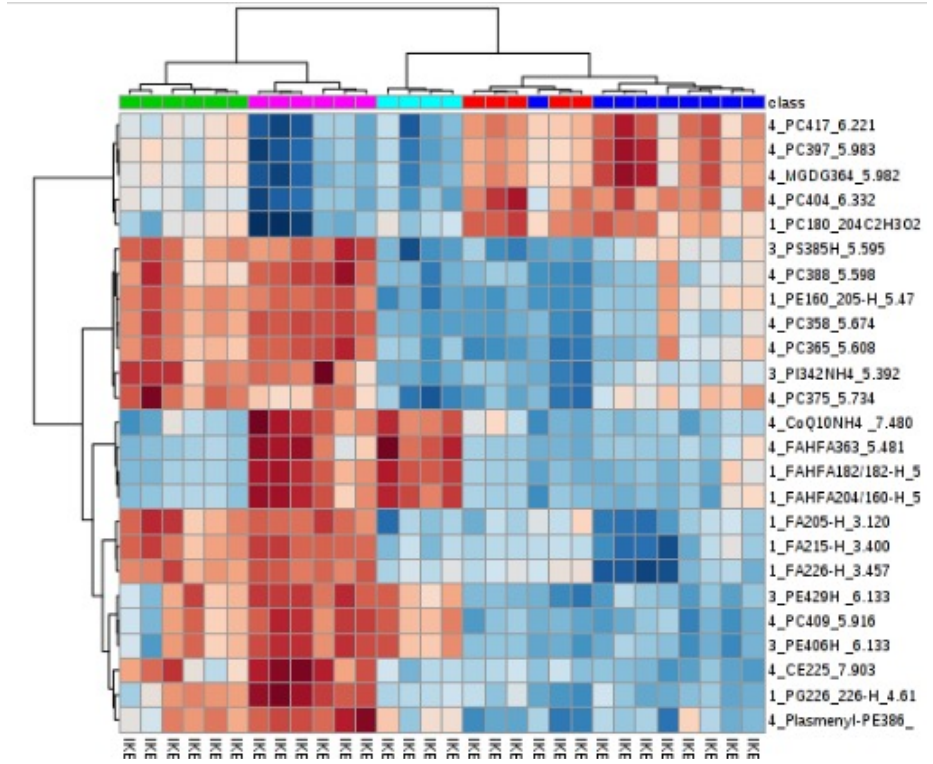


Statistical analysis

Principal component analysis



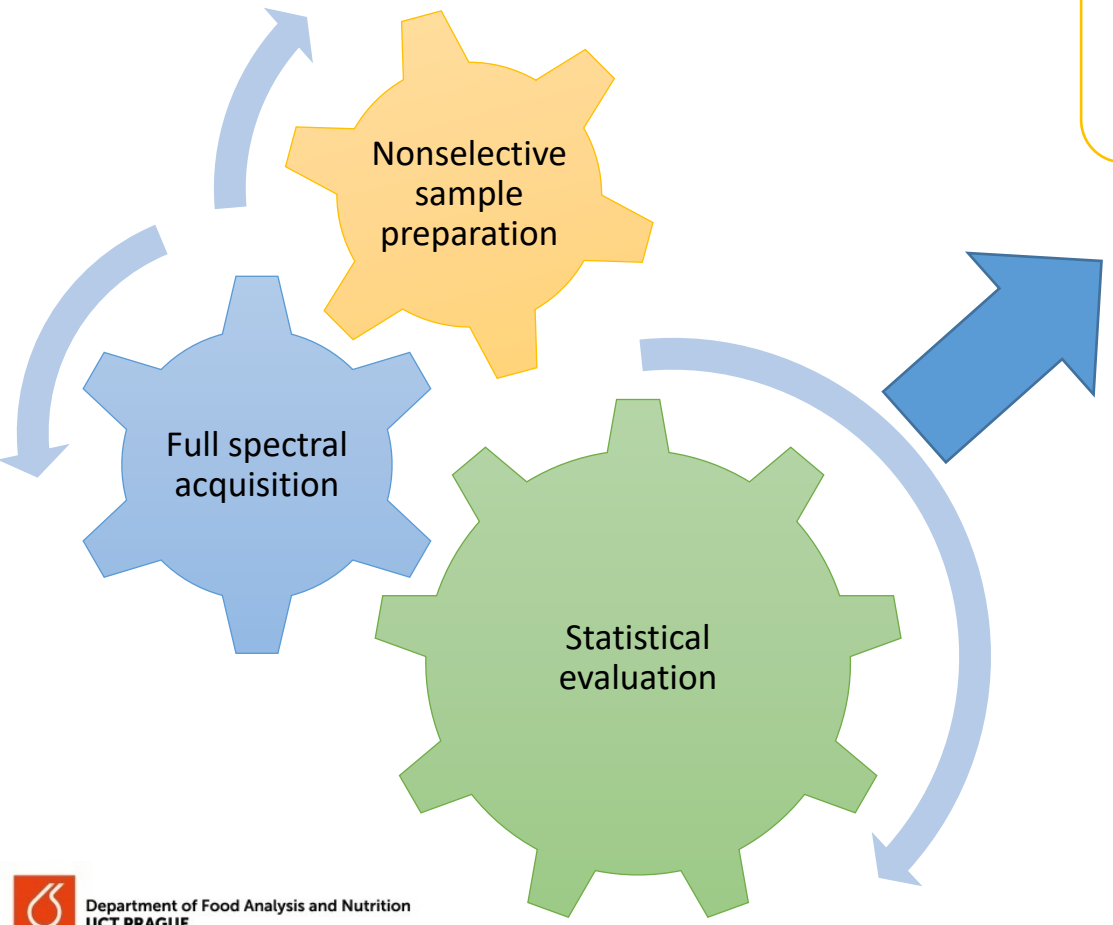
Cluster analysis



Ideal situation

Non-targeted

(10s of samples)



List of „markers“

Targeted

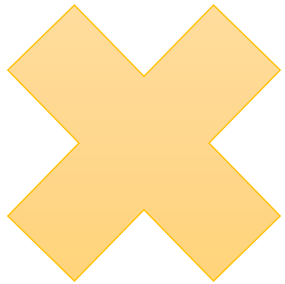
(100s+ of samples)

Ideal situation

Non-targeted

(10s of samples)

- Hundreds of compounds
- Preferring wealth of information over throughput
- Time consuming data processing
- Time consuming statistical analysis and modelling



Targeted

(100s of samples)

- Small number of analytes
- Throughput over data complexity
- Fast data processing
- Rapid statistical analysis and use of models
- More samples to validate the models

Reasons to go to targeted

- Requires less complex instrumentation (cheaper, less requirements for the analyst …)
- Easier to automate
- Is generally more specific and faster -> higher throughput
- The number of variables will be small compared to number of samples -> more robust statistical model
- No need for peak realigning when adding new samples

Conclusions

- Mass spectrometry can be used for food authentication in various strategies
- Recently, fingerprinting is becoming preferred strategy
- Targeted analysis after fingerprinting is still required for routine use