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THE INSTITUTE FOR GLOBAL FOOD SECURITY

Specific case studies looking at authenticity assessment with LC- Q/TOF MS

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Institute for Global Food Security

6th November 2019

Overview

- Introduction
- Rice authenticity project
- Fast methodology for Herbs & Spices authenticity
- Questions

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Mass spectrometry within IGFS

- **Part of Mass Spectrometry Core Technology Unit for Faculty Medicine and Health Life Sciences formed in 2017**

  Moved within new biological Sciences building in June 2019 and with suite of recent instrument including:

  - 3 triple Quad instrument (2 LC and 1 GC MS/MS)
  - 1 single Quad instrument (converted to Ambient MS with DART capabilities)
  - 3 HRMS instrument (2 LC-QToF and 1 LC-QToF converted for Ambient MS with REIMS and DESI capabilities)
  - Sample preparation capabilities with automated derivatisation station and several semi-automated 96-well based instrument to maximise workflow
  - Level 2 biosafety cabinet for clinical samples processing
  - Processing data server with 512GB Ram and 220 TB onboard storage (supported by ECIT), 2 additional storage servers (150 TB) and 4 processing workstation with Progenisis, MPP, LiveID and various MVA software suite

- **Other MS instruments available within IGFS such as IC-ICP-MS(/MS), GC-MS-FID and IRMS**

- **Currently 1 Manager, 3-4 Post Docs, 3 Research Technician/Assistant, 2-3 PhD students dedicated to MS work**

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Mass spectrometry and food integrity

Food safety

Targeted analysis of residues, toxins and environmental contaminants

Mycotoxins

Marine toxins

Pesticides

Antibiotics

Anabolics

Heavy metals

Food authenticity and quality

Fingerprinting analysis and biomarker selection

Food adulteration – herbs and spice, red meat and offal

Food mislabelling - fish, seafood

Food processing – milk

Food quality – meat, poultry

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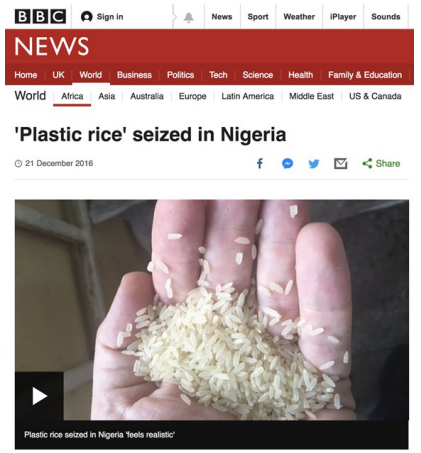
Rice authenticity project

- Rice is the most important staple for more than half the world's population.
- Because of the global economics of rice, it is a prime target for adulteration.
- Asian rice producers have come under fire for making "premium" brands of rice that are essentially "fake."
- Rice Fraud issues -Replace fresh rice with long term stored rice, Bleaching/Polishing/Waxing – Raising physical appearance, GI(Geographical Indications) cheating, Mislabel of functional rice- Selenium rice

The cereal was found in warehouses in Mombasa and there are fears some of it is already in the market.



Nigeria has confiscated 2.5 tonnes of "plastic rice" smuggled into the country by unscrupulous businessmen, the customs service says.

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Overview of the Rice Authenticity Project

Vision

- Create an alliance of key supply-chain stakeholders who share information, intelligence, and globally harmonized laboratory testing practices that support food-fraud risk management.

Goals/aims

- Develop a two-tiered system
- A rapid screening method that can be used in the field to detect and semi-quantify a range of rice frauds
- A more sophisticated laboratory-based method for quantitation and confirmation that employs LC or GC-MS/MS
- Transfer the technology and test methods to our partners in China, Vietnam, and Ghana
- Establish at least one reference laboratory for rice authenticity
- Work to establish globally-harmonized standard test methods, guidelines, and codes of practice
- Conduct a pilot program that will include all of the project partners to evaluate the utility of the system to detect and confirm rice adulteration.
- Work with producers, industry, and governments to reduce/remove technical barriers to adoption.
- Partners will organize and host a series of workshops and training sessions to continue to build capacity and expand capabilities (October 2019)

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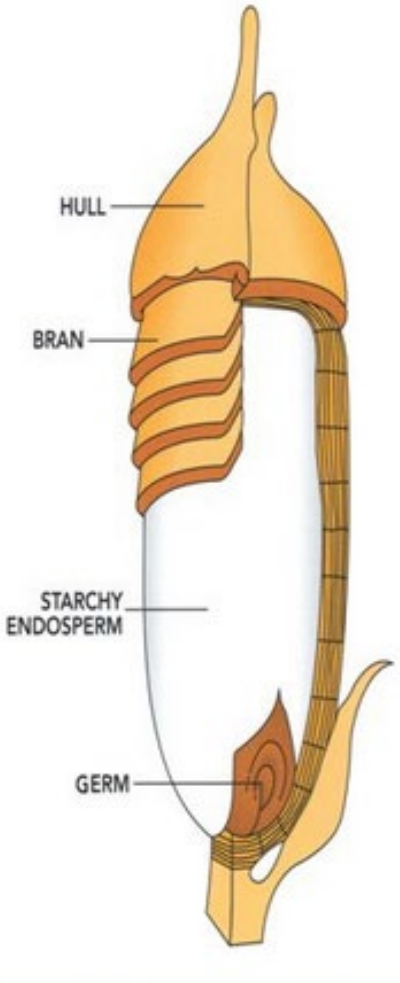
Complexity of Rice Analysis



- Small molecules (vitamins, amino acids and sugars)
- >60% starch content
- Protein
- Lipids mainly in the bran fraction
- Volatiles
- This means a complex matrix requiring
- Robust sample preparation
- Consideration of matrix effects on sensitivity and for long studies
- Different separation techniques

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Compound coverage

MeOH:H_2O(:MTBE)

Organic Phase

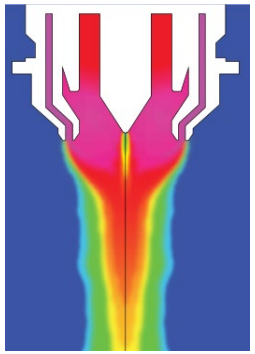
Polar Phase

SFC-QTOF or UPLC QTOF
Lipids

UPLC-QTOF
Secondary Metabolites
RP & HILIC
POS & NEG

GCMS derivatisation primary Metabolites GCMS

Volatiles
GC
Headspace



Proteomics UPLC-QTOF
Protein/Starch

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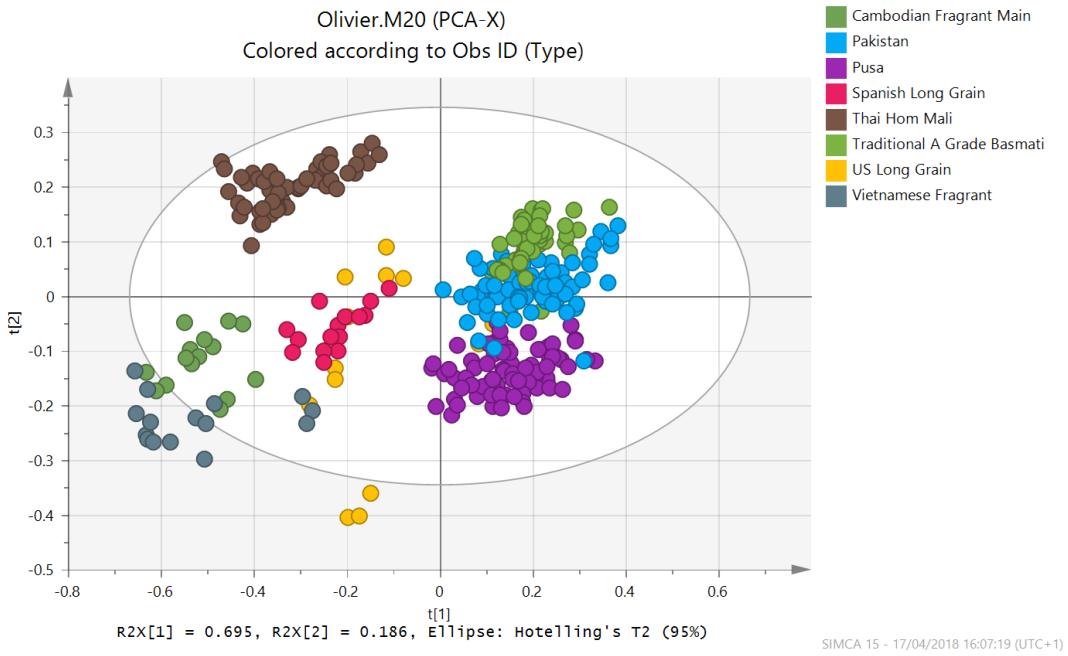
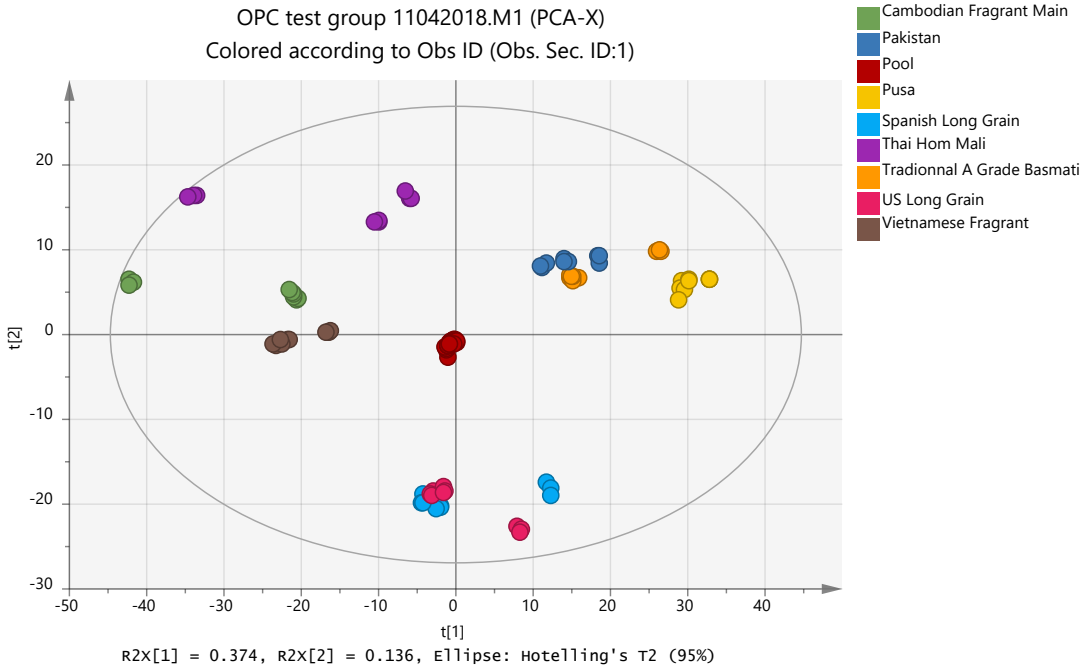
Rice Proof of Concept Project

- Sample set provided by main UK supermarket
- Analysis carried out with portable NIR
- Analysis with LC-HRMS approach



- Comparison between NIR and RP-ESI+
- Extraction 20% MeOH, 20min gradient
- 850 features after filtration one way anova p<0.01
- Separation by GI?



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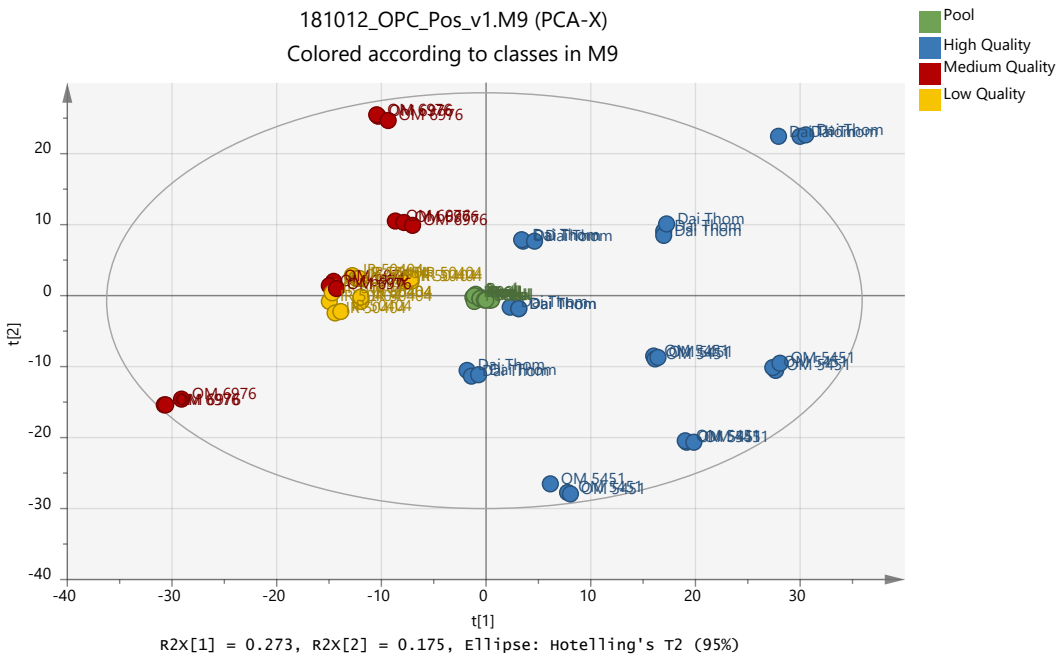
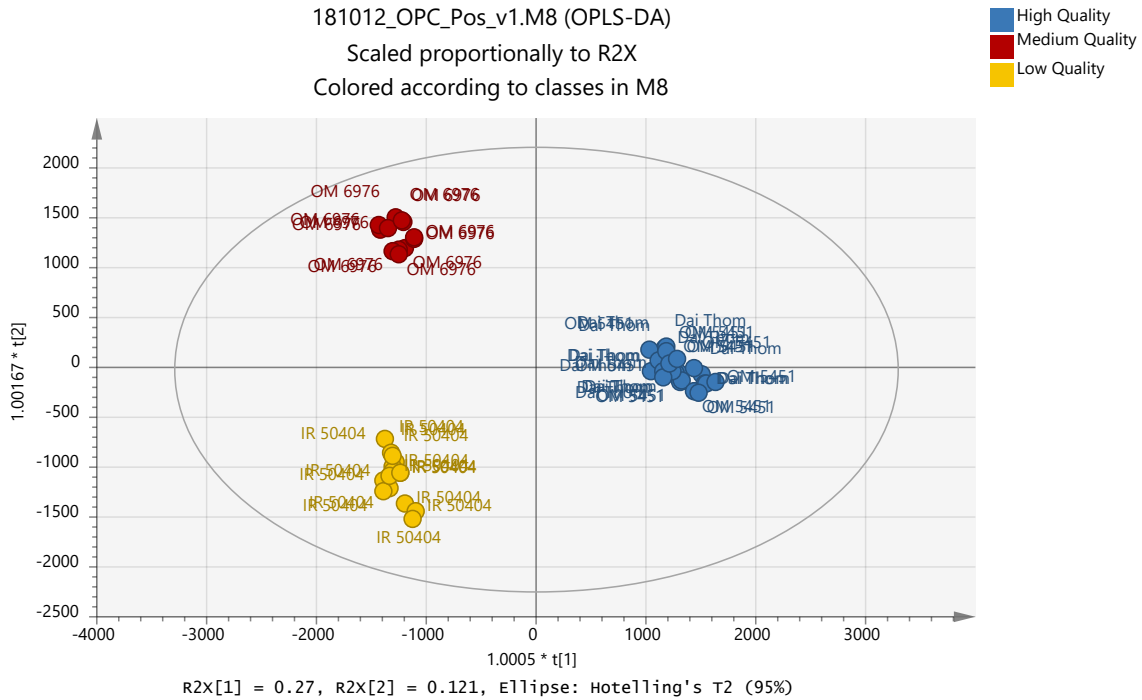
Vietnam Rice Analysis by LC-HRMS





Unsupervised PCA plot UV scaling with 850 features

Supervised OPLS-DA plot for 3 group based on quality attribute

Zorbax Eclipse Plus C18 2.1x50 mm
10 min runtime with water and MeOH with 0.1 FA as mobile phase

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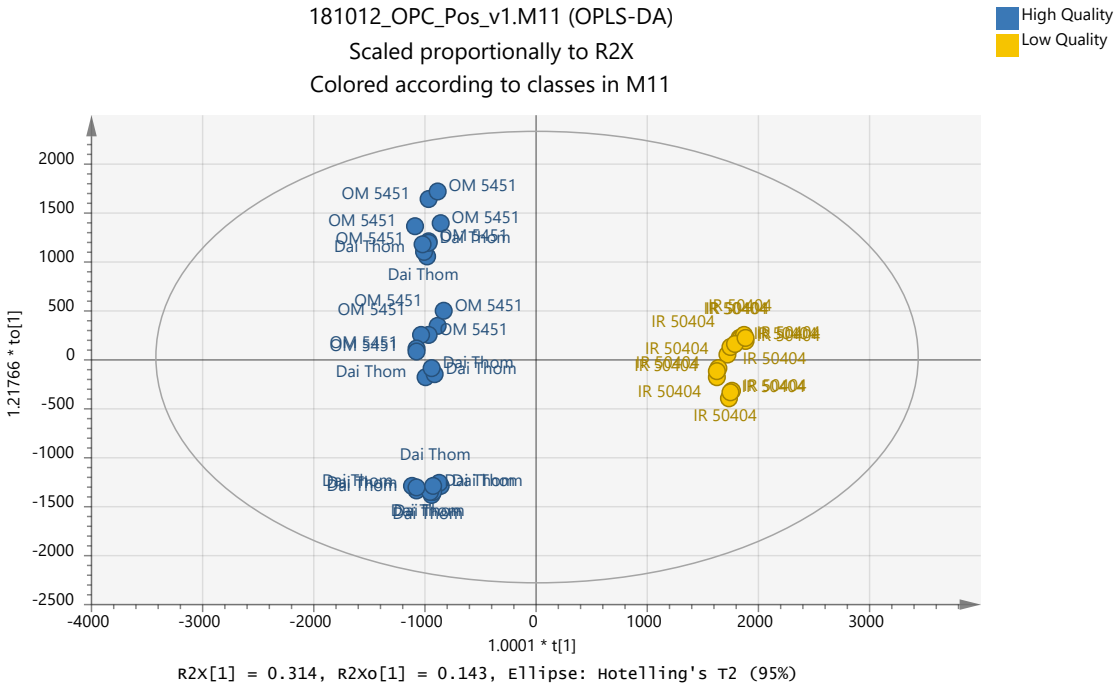
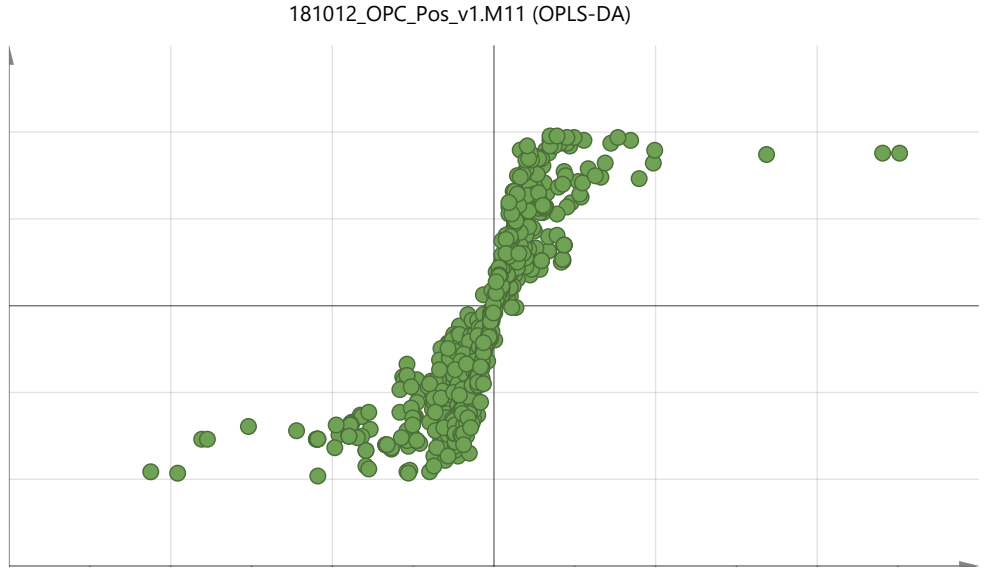
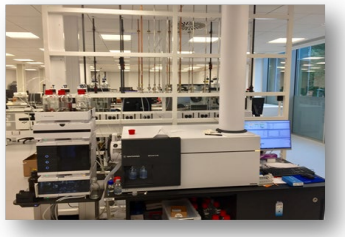
Vietnam Rice Analysis by LC-HRMS





OPLS-DA plot with comparison between 2 group on quality attribute

S-plot showing potential markers of quality to be investigated



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Herbs & spices authenticity

IGFS “Love story” with Oregano: First FTIR method recently accredited for H&S screening for Oregano

Milled herb/spice/adulterant placed directly on the ATR of the FT-IR instrument and the spectra were obtained within 1 minute.

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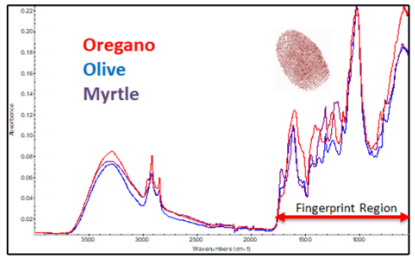
FT-IR Data



Oregano Survey

Global Average of Samples Adulterated= 27%

Country	Samples Adulterated	
South Africa	6/10	60%
Australia	7/12	58%
Denmark	4/10	40%
Canada	6/15	40%
Portugal	2/6	33%
Ghana	2/6	33%
Poland	3/10	30%
USA	12/40	29%
UK	23/82	28%
Italy	11/41	28%
Brazil	2/7	28%
Germany	3/11	27%
Ireland	4/15	26%
Hong Kong	1/5	20%
Thailand	1/5	20%
France	3/15	20%
Norway	3/16	19%
Malaysia	1/6	17%
Belgium	3/24	13%
Netherlands	2/18	11%
Sweden	1/12	8%
Spain	0/6	0%
Vietnam	0/2	0%

Oregano (*Origanum vulgare, Origanum onites*)

Olive (*Olea europaea subsp. europaea)*

Myrtle (*Myrtus communis*)

Cistus (*Cistus laurifolius*)

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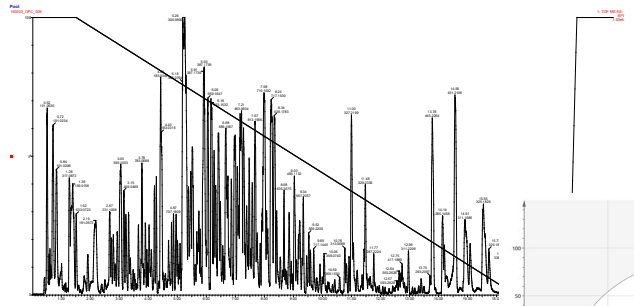
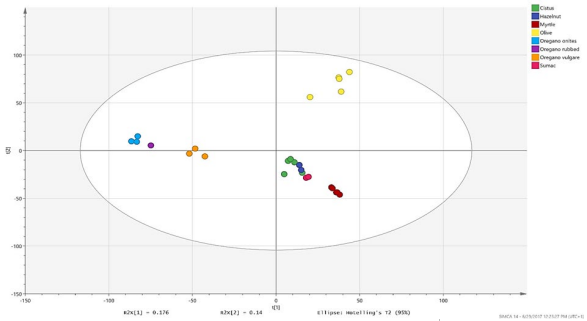
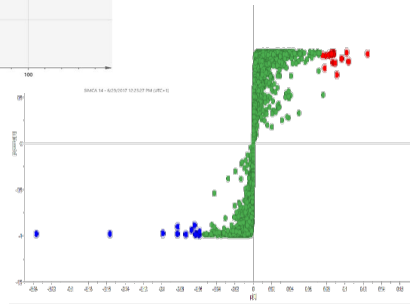
Oregano case study



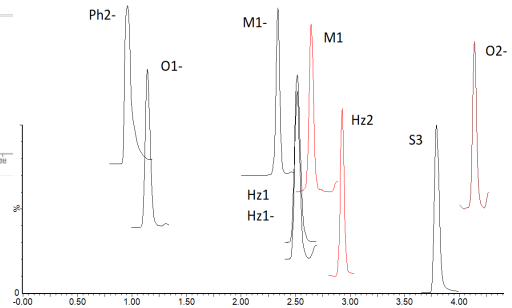
Fingerprinting LC-HRMS



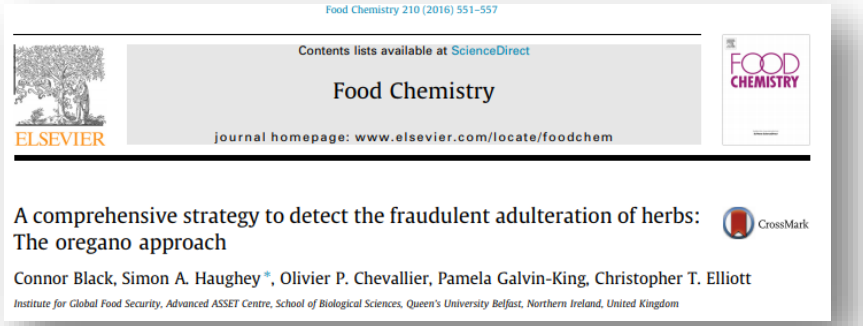
Data processing

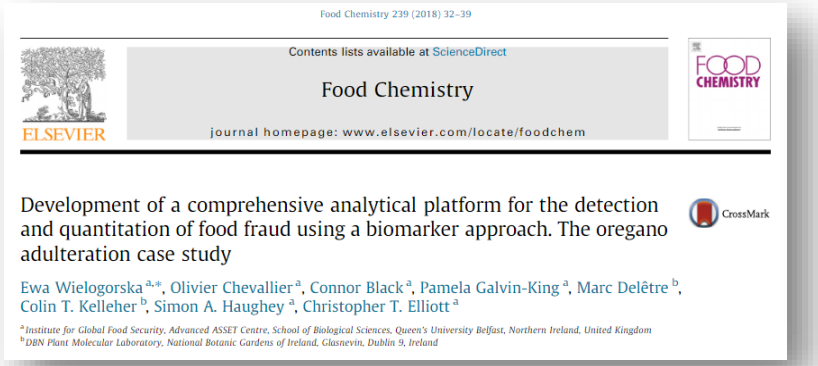


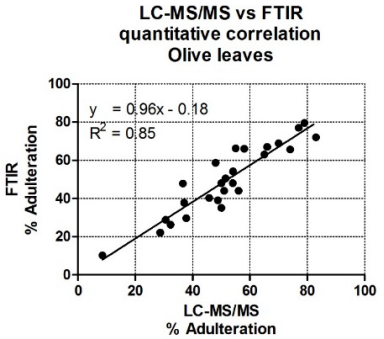
Biomarker selection



Transfer to LC-MS/MS

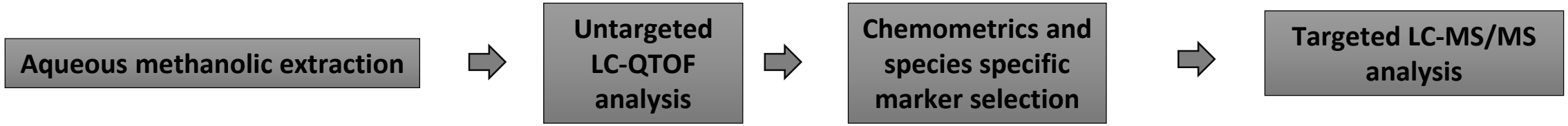






Validation

How to improve workflow?



Current workflow: efficient for "markers" discovery but time consuming

- Sample preparation
- Instrument time
- Data processing
- Data mining
- Instrument access

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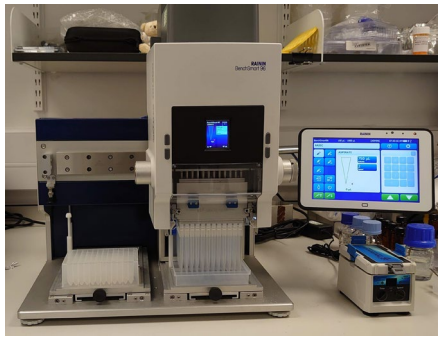
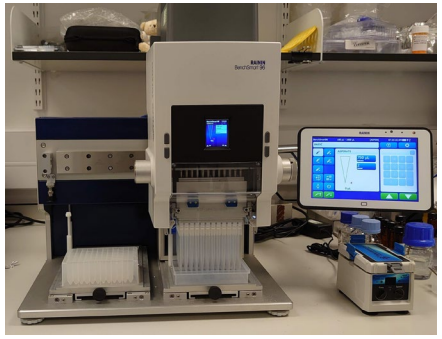
Sample preparation toward automation



Extraction (5 min)

Mixing (15 min)

Centrifugation (15 min)



Transfer (5 min)

Semi automated protocol allows faster, more reproducible sample preparation

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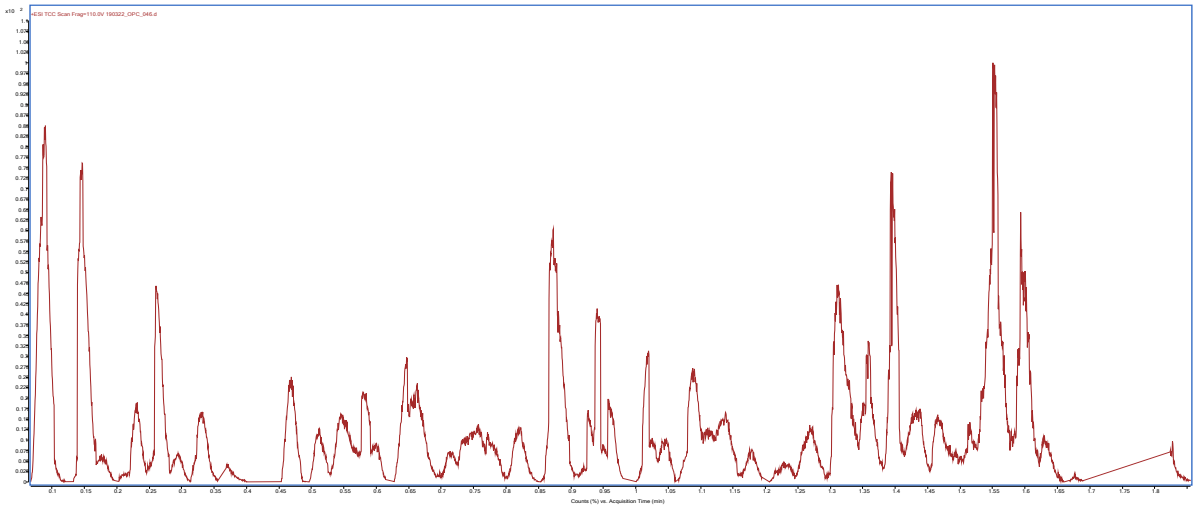
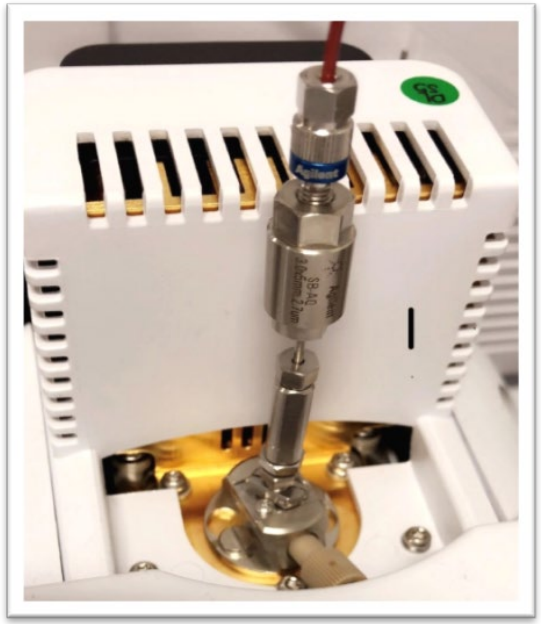
LC-QToF method

- Fast LC gradient with guard column:1.5 mL.min^{-1}
- Fast scan time: 30 spectra.sec^{-1}
- 2 min method:

time	A	B
0	90	10
1.5	1	99
1.85	1	99
1.9	90	10
2	90	10





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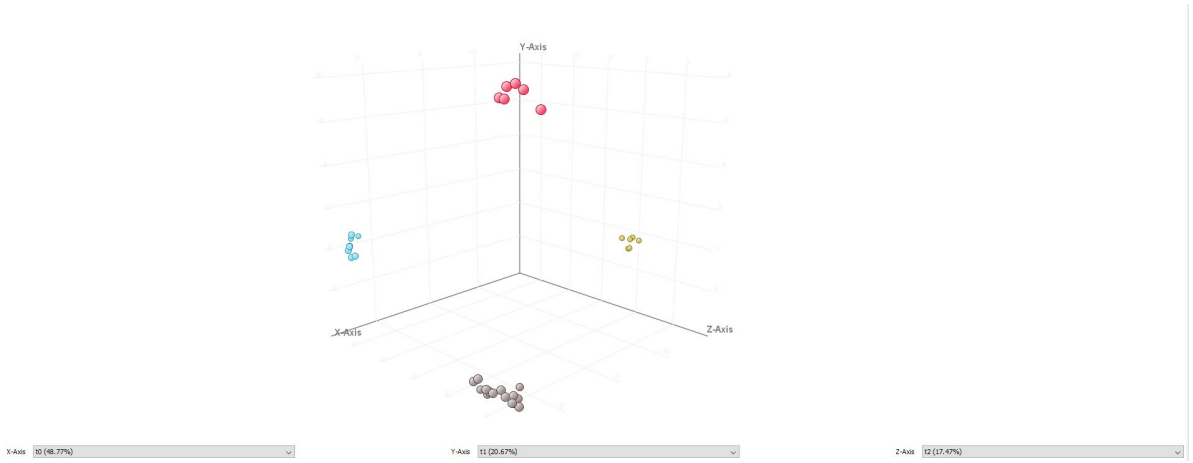
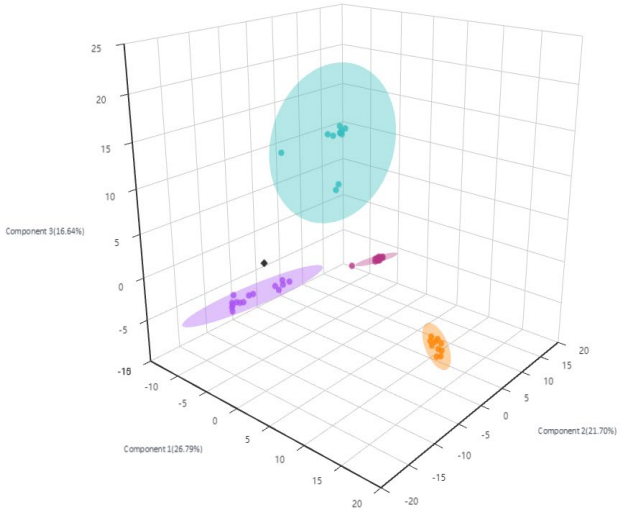
Data processing

- Data processed first with Qual 10 using MFE extraction and converted to CEF
- CEF imported to MPP 15 for filtering and chemometric analysis. PLS-DA model generated
- Transfer to Classifier for analysis of unknow samples





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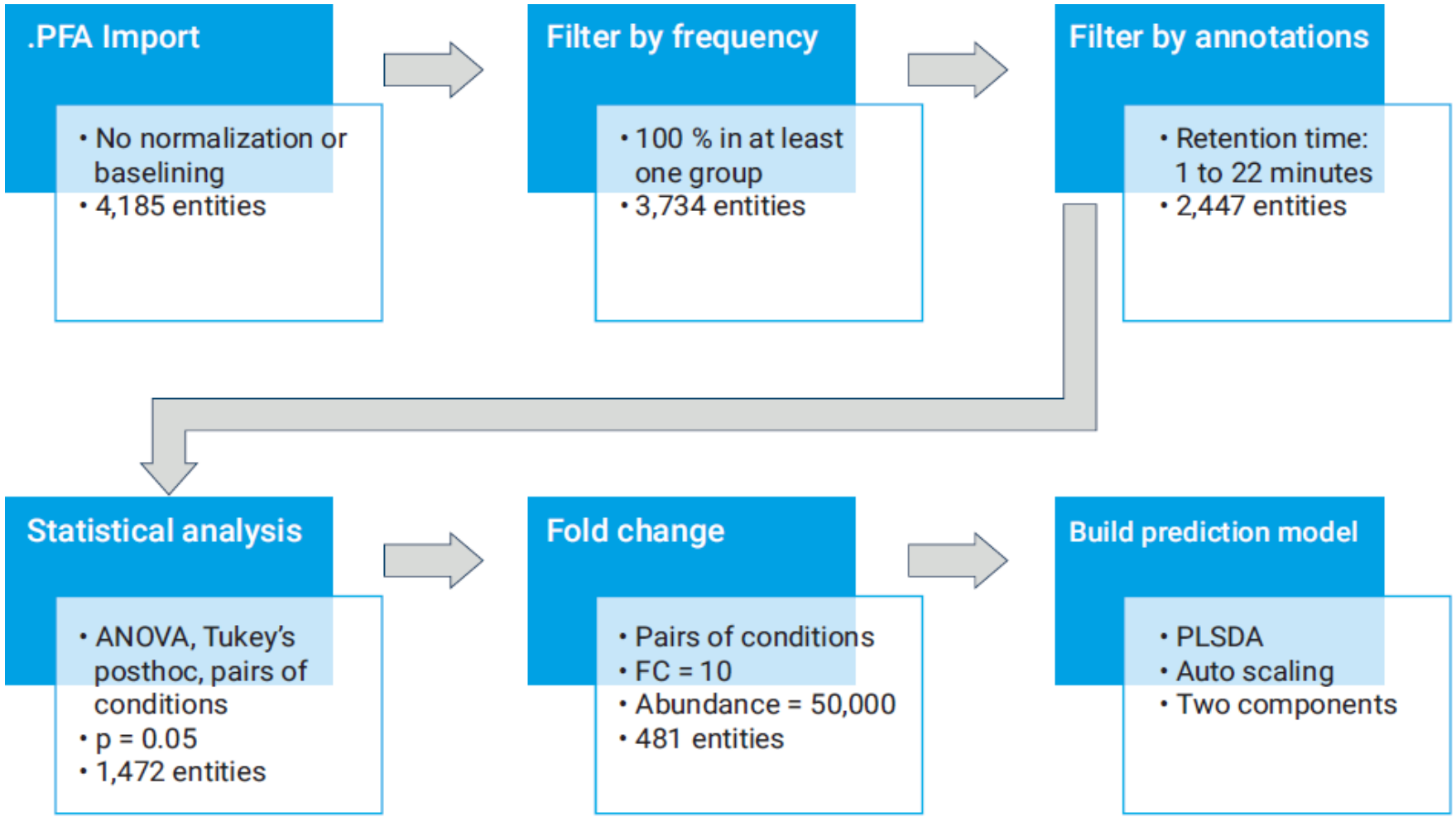
Data processing (alternative)



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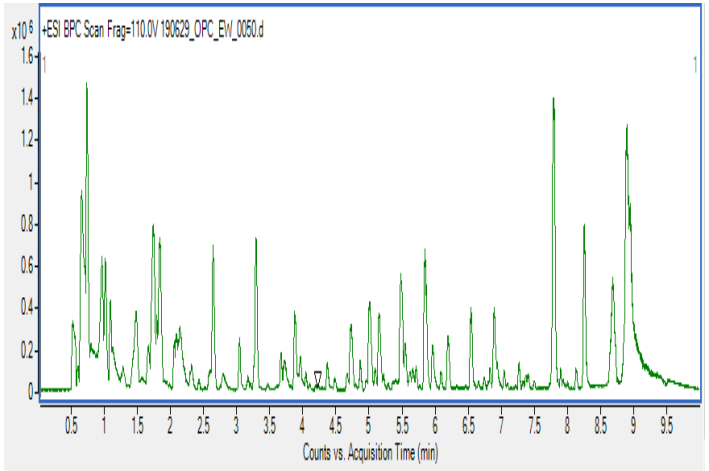
Application on other matrices

Coriander (10/250)
Coriandrum sativum
Oct-March 2018

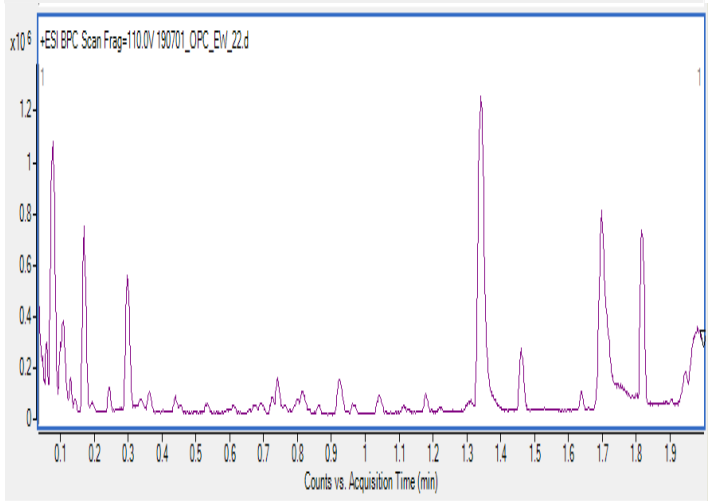
Fennel (10/250)
Foeniculum vulgare
Nov-March 2018

Cumin (10/250)
Cuminum cyminum
Nov-March 2018

10min gradient
Luna Omega Polar C18



2min gradient
Polar C18 Guard



Extraction: 50mg of ground seeds + 2mL H2O/MeOH (1:1), 500µL dried down and reconstituted in 300µL H2O, 200µL passed through a Costar Spin-X filter

Work carry out by Dr Ewa Wielogorska (Chemjet Researcher, UCT Prague)

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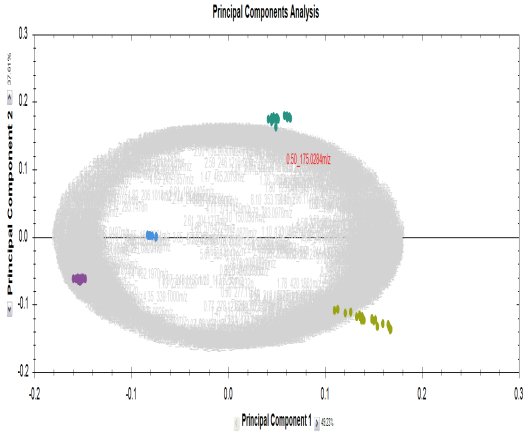
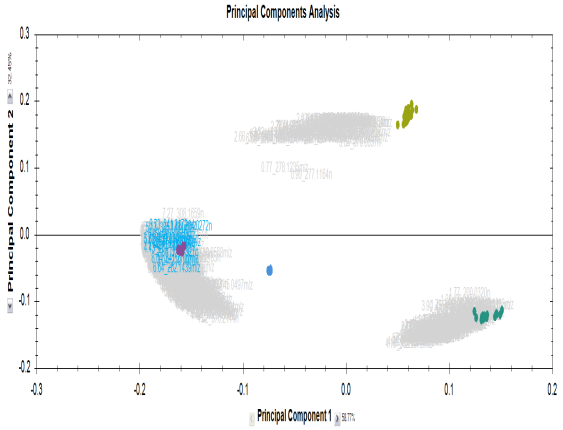
Markers detection abilities

10 minutes

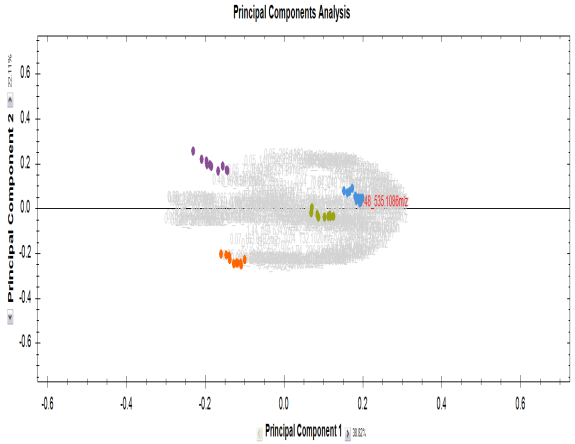


All features (1946)
PC1+2 89%

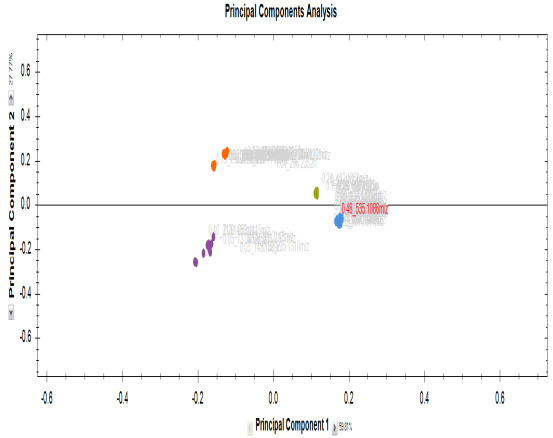


Exclusive markers (472)
PC1+2 91%

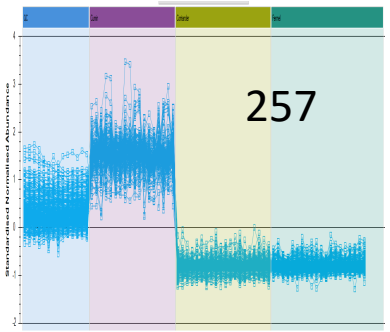
2 minutes

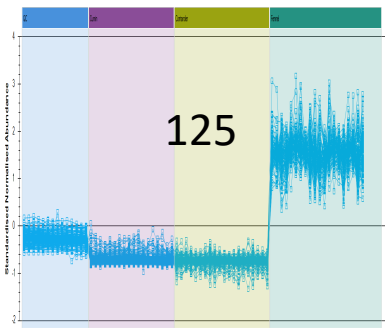


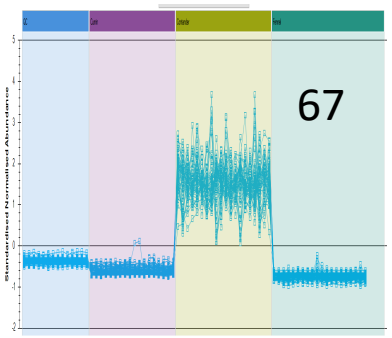
All features (255)
PC1+2 61%

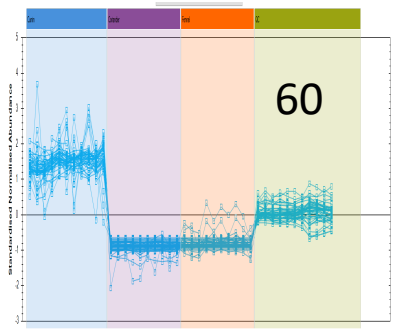


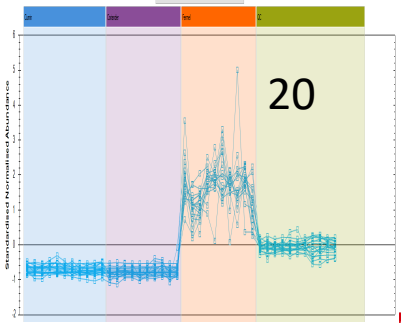
Exclusive markers (87)
PC1+2 88%

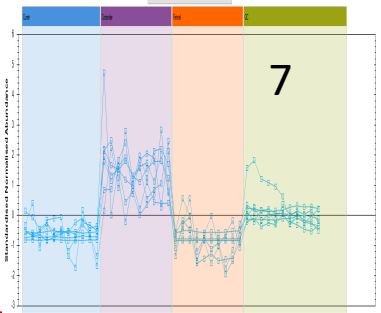












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Conclusion

- LC-Qtof system essential in all authenticity work
- Markers discovery work
- Screening work
- New fast methodology with chemometric model building

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