Rapid analysis of metabolomic fingerprints using FTMS instrument coupled with chemometric tools to highlight pesticide exposure status

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SCIENCE & IMPACT

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  - ✓ Chemometric methods (ICA & ICA-DA)
  - ✓ Metabolite annotation
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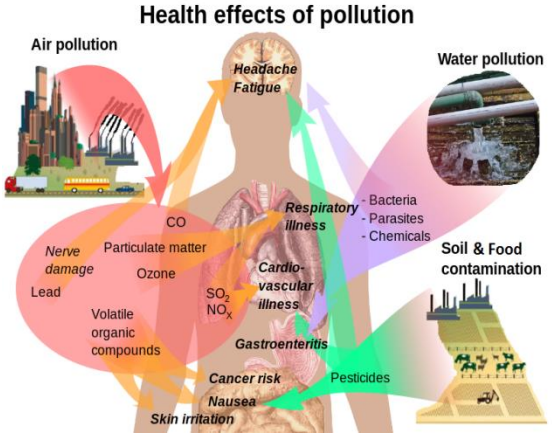


Many of the questions faced by life science researchers involve large number of samples

Interesting method to perform high-throughput analysis :
DIMS (Direct Introduction Mass Spectrometry)
combined with high-resolution mass spectrometry

Data analysis

large number of samples analyzed

complexity of acquired mass spectra (hundreds to thousands of signals)

Bioinformatics, chemometrics and statistical treatments

detection of exposure biomarkers

Han J. et al; Metabolomics: Official journal of the Metabolomic Society; 2008; 4; 128-140

Pre-processing:
Filtering,
Normalization …

Develop a simple and fast chemometric procedure for the treatment of spectral data obtained by **DI-HRMS** metabolomics approach
New supervised chemometric tool Independent Components Analysis-Discriminant Analysis **(ICA-DA)**

Processing:
Chemometric methods

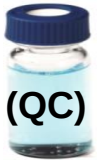
Highlight the metabolic disruptions induced by exposure to pollutants present in the environment or food

Metabolites putatively annotation
Database
Correlation coefficients

Analytical method: **Direct injection without LC coupling (FIA mode)**

Samples: urine of farmers exposed to two types of pesticides Isoproturon or Captan

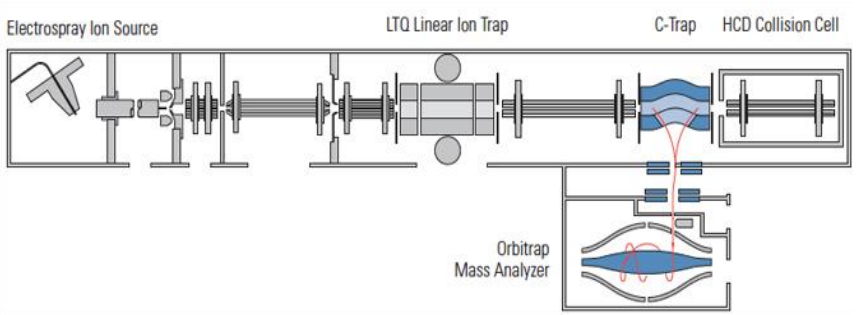
Quality Control



Assess the quality of the data produced

= Pool of samples + Standard solution:
Oxytetracycline (PM 460)
Isoniazide (PM 137)
PhIP (PM 224)

Different dilution factors, in H_2O/MeOH (50:50): 1/50; 1/100; 1/250; 1/200; 1/500



Mobile phase :
H_2O/MeOH (50:50)
+0.1% HCOOH in ESI (+)
Flow rate: 100 µL/min

Electrospray Ionisation (ESI) in (+/-) ion mode
V_{inj}: 10 µL of sample

LTQ-Orbitrap(XL-ETD)
Resolution:
60 000 @ m/z 400
m/z: 50-1000
Cycle time:3 min

Makarow A. et al; J Am Soc Mass Spectrom; 2006;17: 977-982

1.Pre-processing

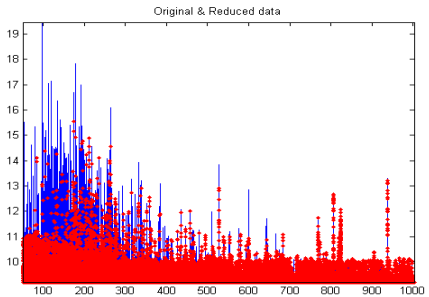
Raw data
Convert into net.CDF
Import into matlab

Matrix of data
(selected scans)

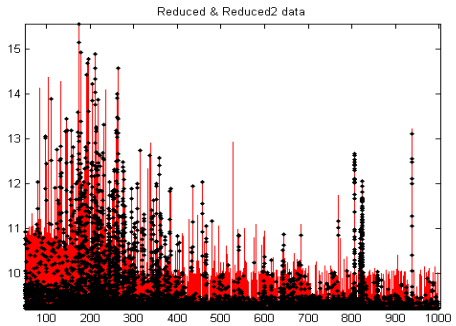
Remove variables with signals present in at least 2 blanks

Data filtration

Remove variables with no signal in QCs



PQN (Probabilistic Quotient Normalization) transform using mean of QCs & Pareto scaling

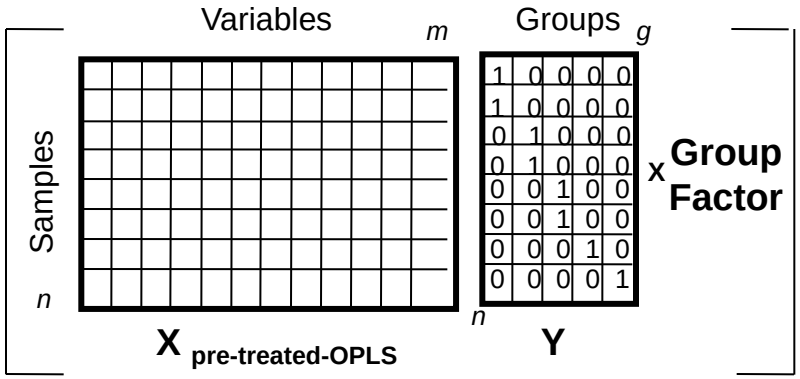


Orthogonal correction:
OPLS
remove variations from the input data set X that are not correlated to the response set Y

2. Chemometric methods: ICA & ICA-DA

a. Selection of variables

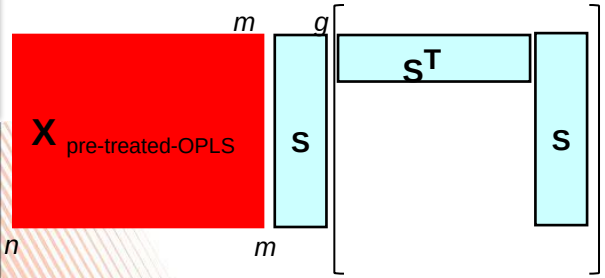
Input



Optimal number of LVs and ICs for the OPLS and ICA-DA is determined simultaneously: maximizes the value of the **F of Fisher** associated with the Wilks' lambda

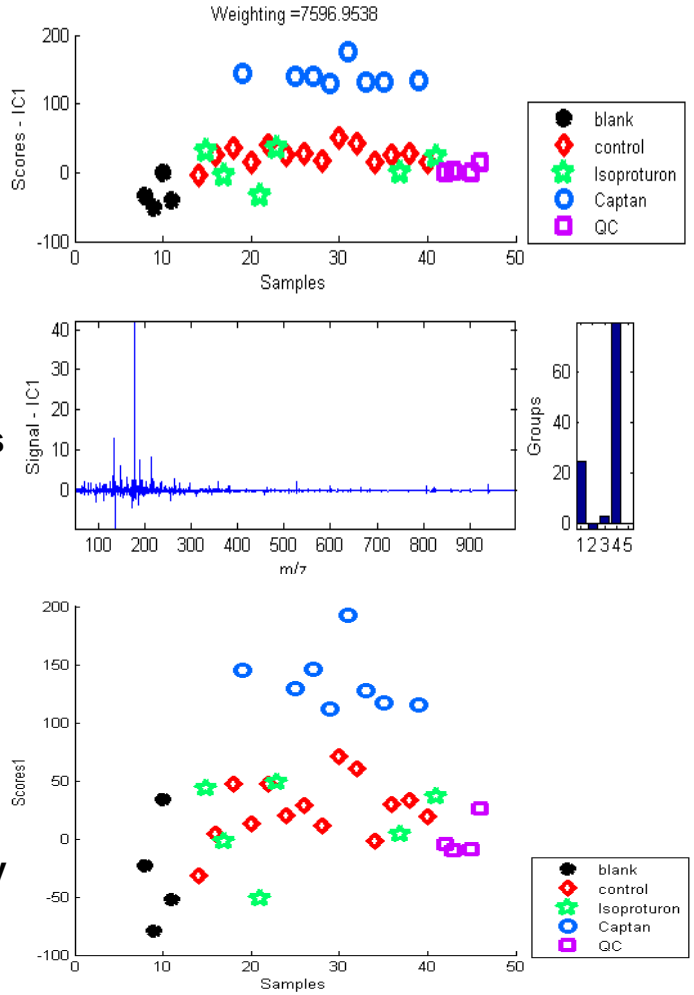
ICA

Selection of variables by groups
Concatenating matrix spectra with groups



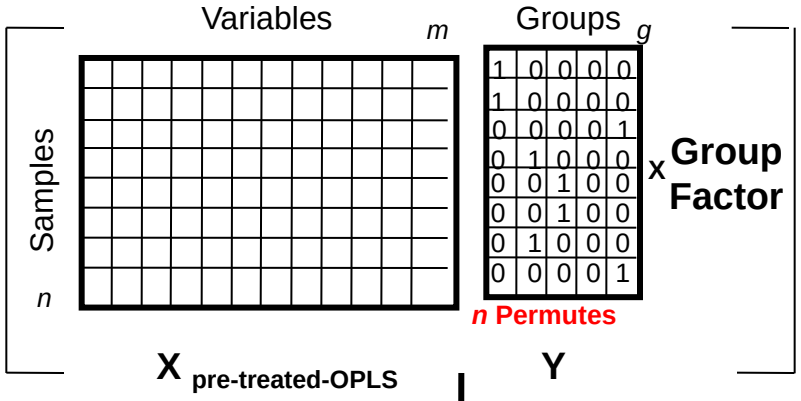
Prediction of groups by selected variables
Considering matrix spectra only

Output



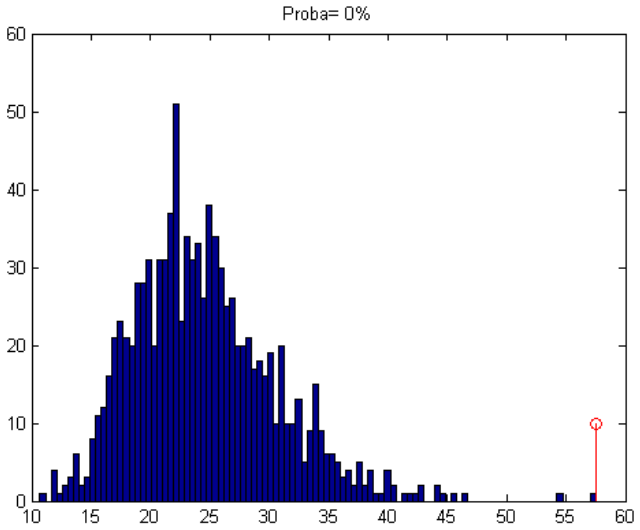
2. Chemometric methods: ICA & ICADA

b. Permutation



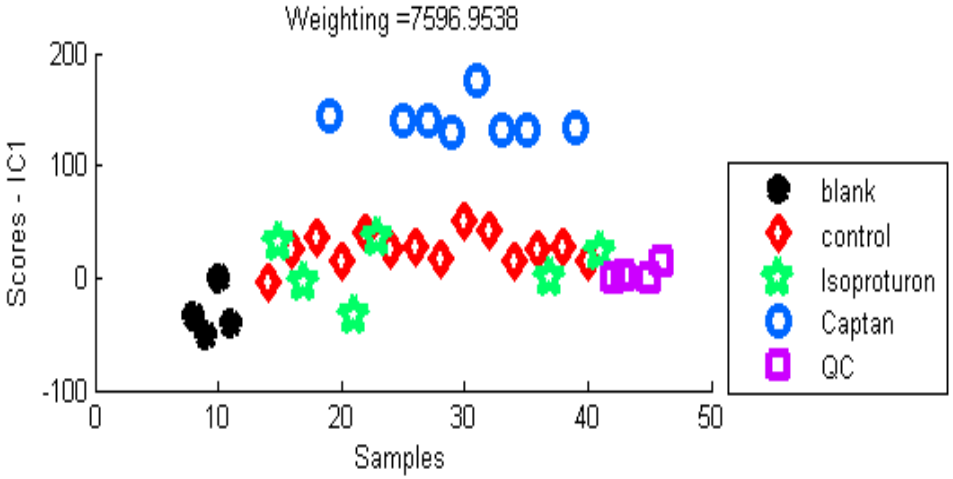
ICA calculation repeated 999 times

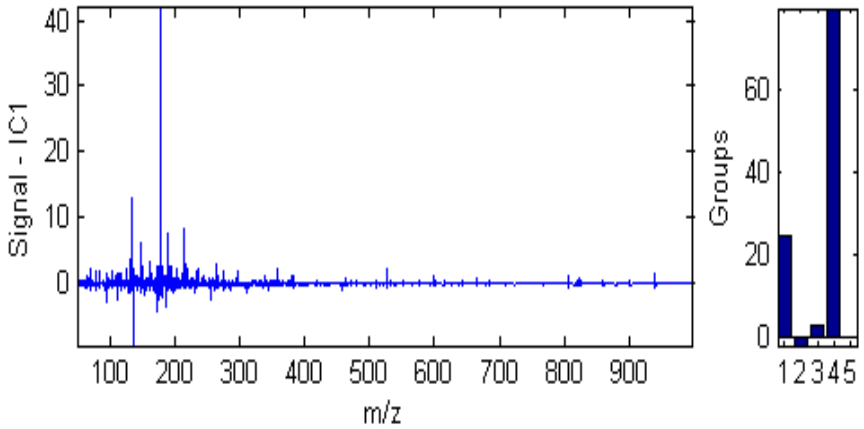
Wilk's Lambda & F-ratio (F of Fisher) calculation



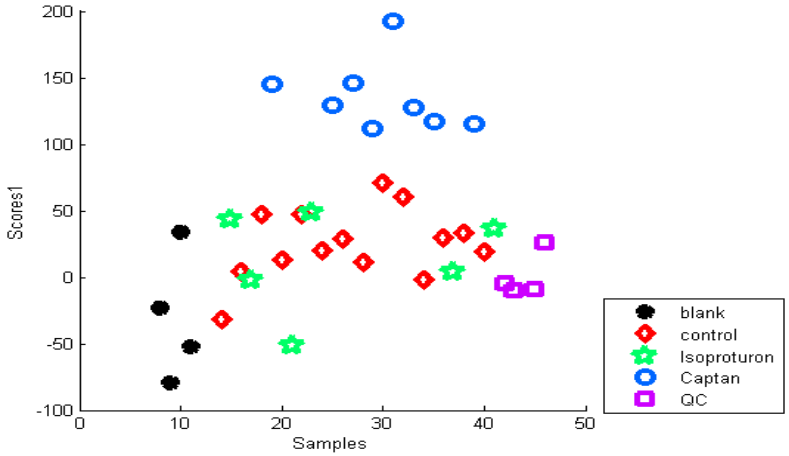
1. ICA-DA results in ESI(-)

Concatenating matrix spectra with groups





Considering matrix spectra only



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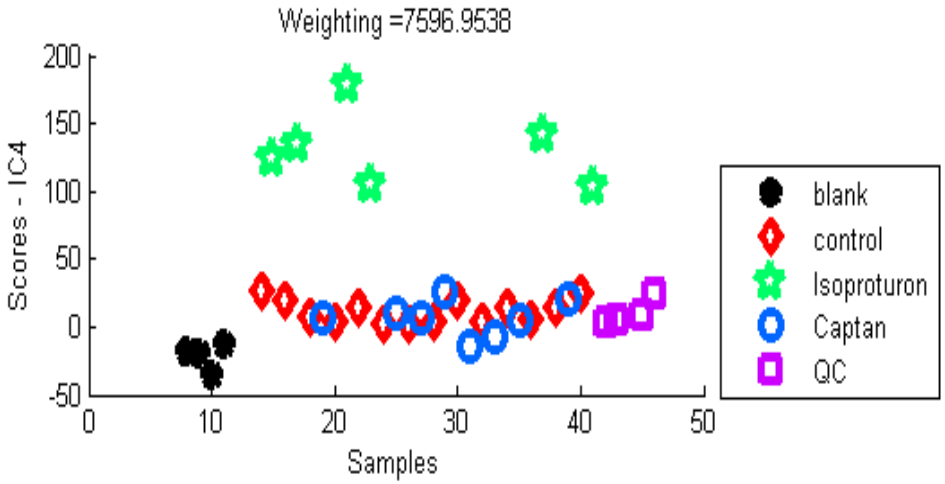

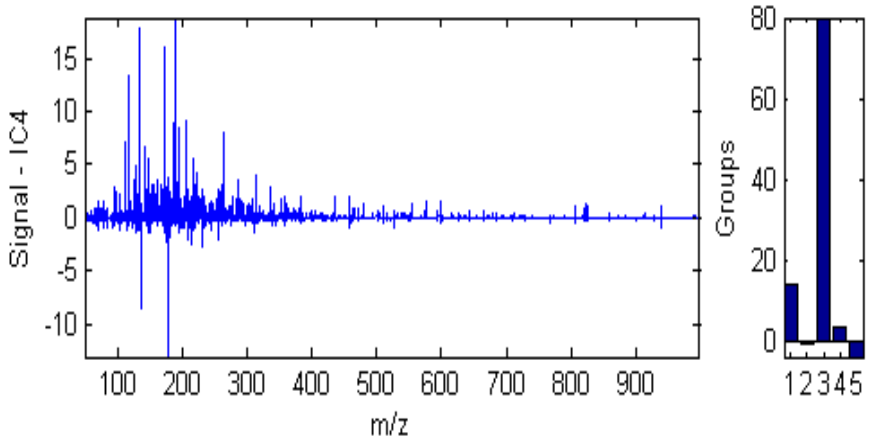

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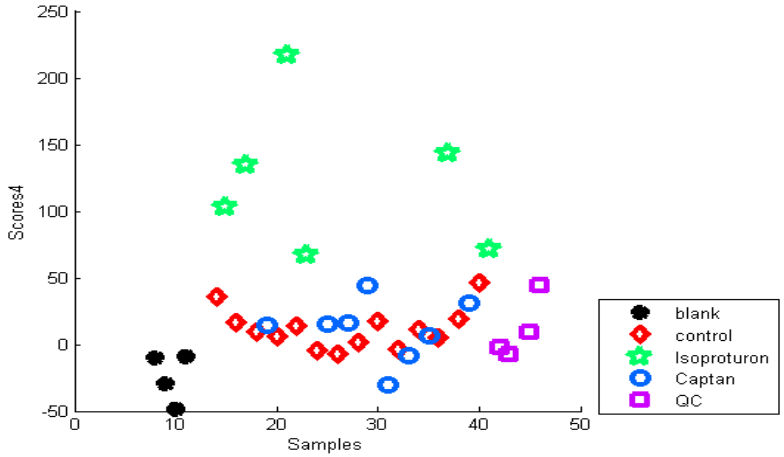

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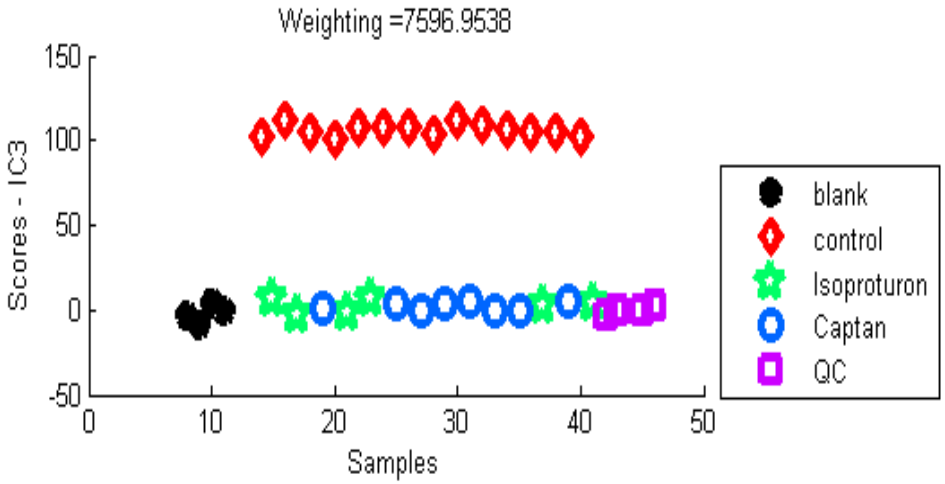
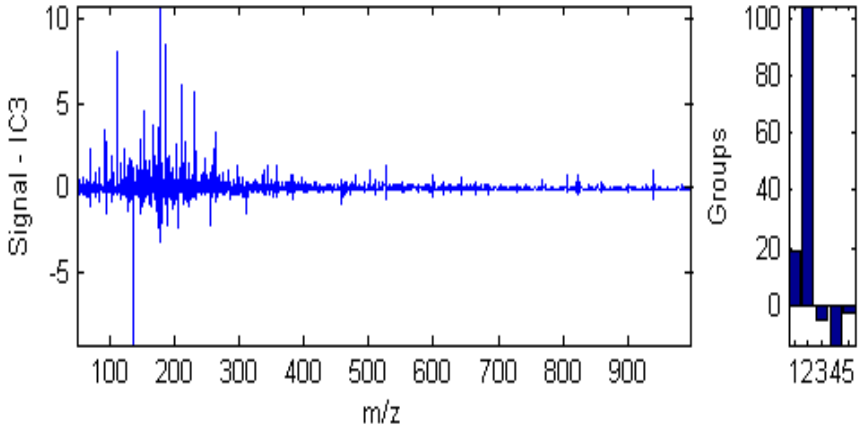
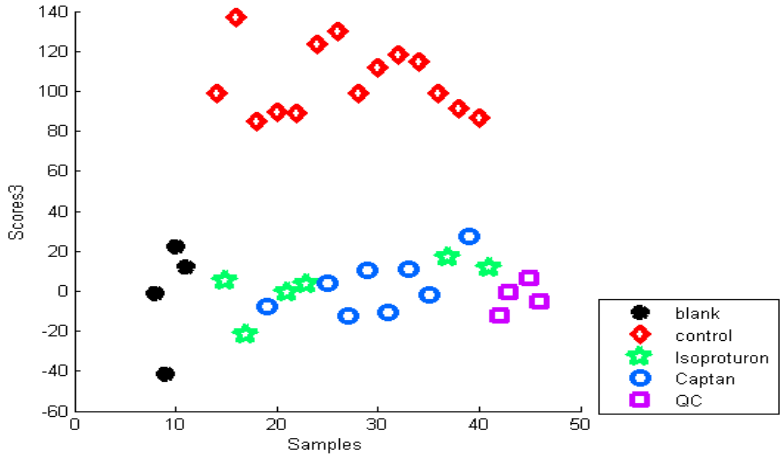
Concatenating matrix spectra with groups



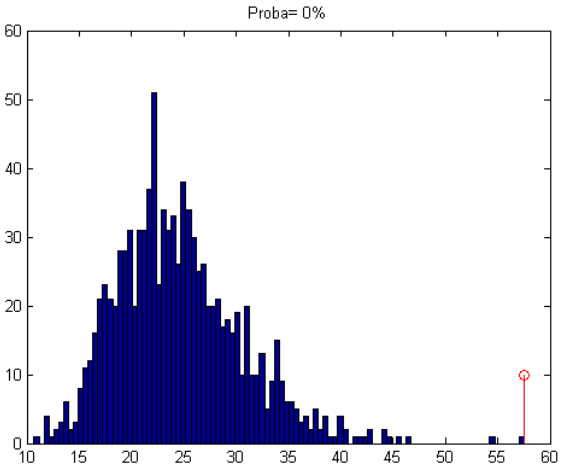


Considering matrix spectra only



2. Validation of ICA-DA

a. Wilk's Lambda & F-ratio (F of Fisher) calculation



b. Comparison with a commonly-used method:

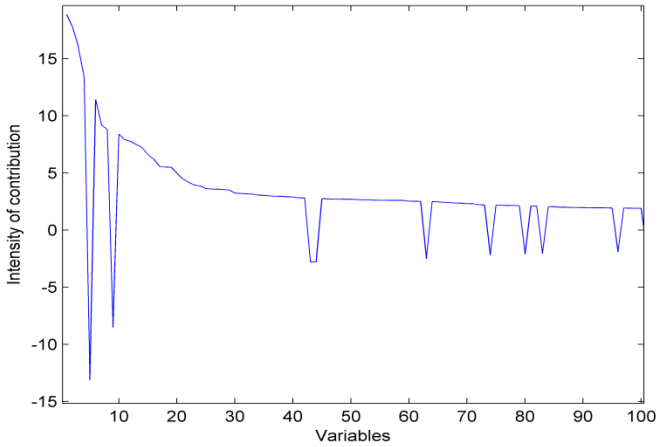
Partial Least Squares - Discriminant Analysis (PLS-DA)

- Only a partial discrimination of isoproturon and captan groups
- No discrimination of control groups in both modes data
- Lower correct classification rates

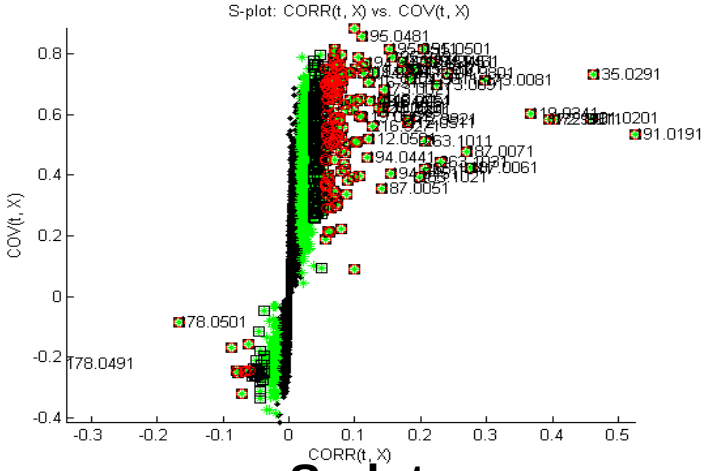
For more detailed consult the poster 18

3. Metabolite annotation:

a. Discriminant variables selection



Variables with the highest absolute value



S-plot

b. Database annotation

Mass list :

Variables with very different contribution coefficient intensity between isoproturon, captan and control groups are preserved 38 in ESI(-) data and 21 in ESI(+) data

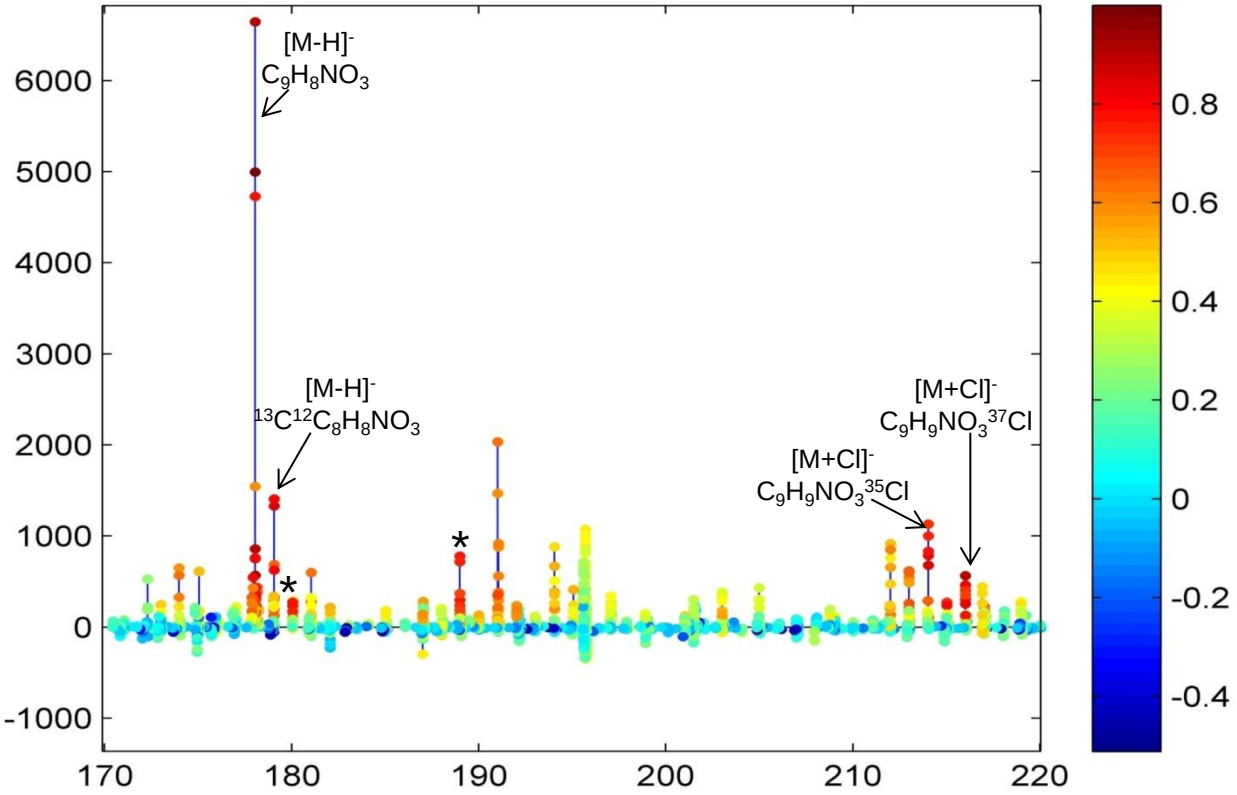
Queries in DB: **HMDB** and **KEGG**

Putative annotation

2. Metabolite annotation :

a. Correlation coefficients

Improve putative annotation: correlating discriminant variables associating the putative identified metabolite species with redundant signals such as the isotopic peaks (e.g., ^{13}C, ^{34}S and ^{37}Cl)



- ICA-DA provides a clear discrimination of all groups whereas PLS-DA provides only a partial discrimination of human exposed to isoproturon and captan groups

- ICA-DA provides higher correct classification rates

- Around 60 discriminant variables were detected
  45 of them were putatively annotated

ICA-DA method facilitated the detection of variables to discriminate the different groups and demonstrated the feasibility of such a direct introduction approach in metabolomics studies

Validation of putative annotations (MS/MS experiments) → Structural identification of metabolic biomarkers

Application of ICA-DA on other types of data

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Pascal Gauduchon
Pierre Lebailly

Additional and interesting results are presented by the poster 18