University of Glasgow

hiRACE
High Resolution Analytical Chemistry for Environmental Engineering

Workflows for Signature Analysis by Gas Chromatography Coupled with Mass Spectrometry.

Examples for Description of Engineering Processes.

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Signature Analysis: Non-Targeted Approaches to Complex Samples

Non-targeted analysis (NTA) is changing chromatography and mass spectrometry. In contrast to conventional analytical methods that focus on the targeted identification of specific compounds in samples, NTA offers a holistic view of complex samples, exploring the entire "chemical space." Enabled by cutting-edge hardware and data science and initiated by biological applications, the rapid growth of this technique is also significantly shaped by its applications in environmental sciences.

Just as an individual's signature evolves with time and stressors, so do the chemical profiles in environmental samples. Instead of a "chemical fingerprint," we should call it a "chemical signature." Think of NTA as signature analysis – the path to extracting unique insights from samples that targeted analysis can't uncover. NTA has the potential to elucidate the mechanisms of the transformation of complex environmental systems and to isolate markers for these reactions.

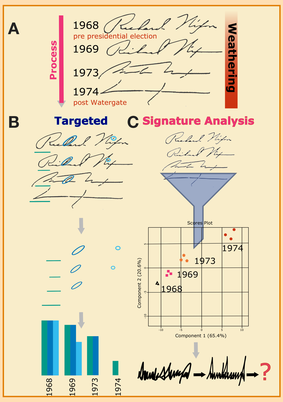


Figure 1- To grasp the value of signature analysis in the context of environmental samples, let's draw a parallel with handwritten signatures. In this illustration, we trace the evolution of signatures from two U.S. presidents, namely R. Nixon and D. Trump.
A- Nixon's signature seems to bear the marks of his time in office, marked by the Watergate scandal and the subsequent impeachment proceedings leading to his resignation.
B- However, if we restrict our analysis to specific graphological elements, like the shape of the capital 'R,' the loop on the 'h,' and the dots on the 'I,' we inadvertently overlook other significant characteristics, rendering the data unusable for further extrapolation.
C- Conversely, when we undertake a holistic examination of the entire signatures, we open the door to the identification of new statistical markers and the development of transformation models. The model, statistically constructed from R. Nixon's signatures, offers the potential to predict the impact of impeachment on D. Trump's signature.

Signature Analysis to Describe the Gasification of Coal and its By-Products

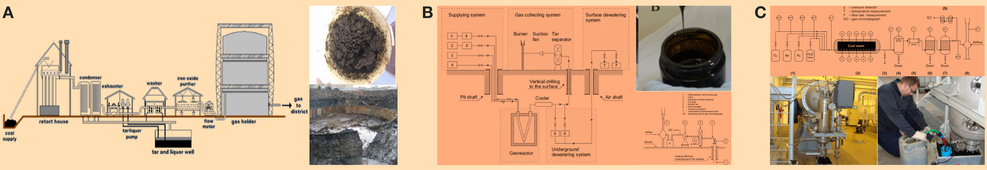


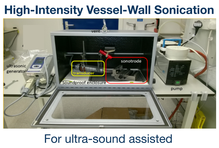
Figure 2- Processes of coal transformation investigated and the types of samples analysed.
A- Manufactured Gas Works and coal tar and tar contaminated soils.
B- Coal Gasification in Barbara Mine, Poland and coal tar. Diagrams from Wiatowski *et al.* (2019).
C- Ex-situ high pressure underground coal gasification (UCG) reactor and processed waters. Diagrama and photos from Pankiewicz-Sperka *et al.*

Sample preparation
•Exhaustive extraction,
•Increase of the number of features through derivatisation for polar compounds.

Analysis
•Optimisation of the chromatography for maximal use of chromatographic space.

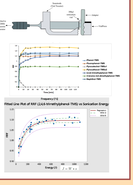
Example- Exhaustive liquid-liquid extraction of processed waters from UCG.

High-Intensity Vessel-Wall Sonication

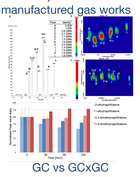


For ultra-sound assisted
•Liquid-Liquid Extraction
•Dissolution
•Solid Liquid Extraction
•Derivatisation

Example- Derivatisation of coal gasification tar.

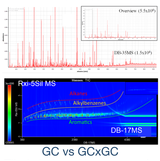


Example- Tar from manufactured gas works



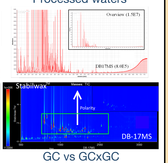
GC vs GCxGC
Reverse phase provides better resolution of aromatic

Example- Tar from coal



GC vs GCxGC
Tiled elution of isomers and homologs.

Example- Processed waters



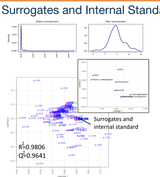
GC vs GCxGC
Specific column combination for phenols.

Chemical Signatures → **Environmental/Geo Engineering Processes** → **Altered chemical Signatures**

Enables to describe a process as a whole not by individual compounds.

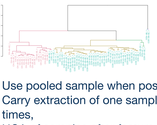
QA/QC
- Monitor sample preparation reproducibility- pooled samples, surrogates,
- Monitor chromatographic shifts- pooled samples, internal standards,
- Multivariate analysis of replicates and check samples to understand error.

1- Surrogates and Internal Standards



- Quantitative check of recovery,
- Run PCA/PLS-DA with "all features" ,
- Check that surrogates and internal standards are near (0,0) on loading plot.

2- Reference Sample



- Use pooled sample when possible,
- Carry extraction of one sample 5/6 times,
- HCA of samples after feature selection: closest neighbour should be duplicate.

QA/QC
- Capture heterogeneity of samples- subsampling theory, experimental replicates,
- Monitor sample preparation reproducibility- pooled samples, surrogates,
- Monitor chromatographic shifts- pooled samples, internal standards,
- Multivariate analysis of replicates and check samples to understand error.

Sample preparation
- Exhaustive extraction,
- Increase of the number of features through derivatisation for polar compounds,
- Thermal desorption and pyrolysis for applications to (micro)plastics and organic matter.

Analysis
- Optimisation of the chromatography for maximal use of the chromatographic space,
- Increase resolution and sensitivity with (multi)detection: HRMS, VUV, soft

Data Processing
- Data cleaning to maximise the number of features (e.g. wavelet analysis),
- Features picking (e.g. peak searching or binning),
- Data reduction (F-ratio, Regions of Interest, wavelet compression),
- Further data treatment: replacing zeros, saturated peak correction,

Data Exploration
- Machine learning to establish relationships between features and/or samples,
- Trend assessment,
- Marker discovery.

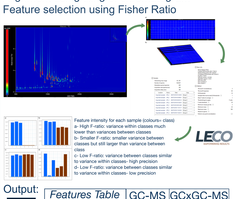
Correlation and Integration
- Integration with other omics- high throughput sequencing, metagenomics, lipodomics, proteomics,
- Integration with QSAR or mechanistics models,
- Integration with toxic assay- Effect Directed Analysis.

Annotation
- Features into compounds
- Identification by comparison to library
- Componentization

Data Processing
• Features picking
• Data Reduction

GC-MS data:
Data processing workflow based on available metabolomics tools and methodologies

GCxGC-MS data:
Alignment using tiling of chromatogram
Feature selection using Fisher Ratio



Output:

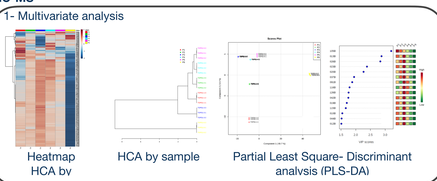
Features Table	GC-MS	GCxGC-MS
Tar from coal gasification	245	2879
Procesed waters from UCG	146	896

Data Exploration
•Machine learning to establish relationships between features and/or samples,
•Trend assessment,
•Marker discovery.

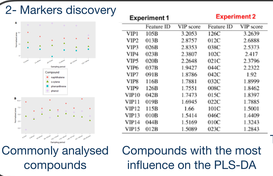
Example of time series of processed water from two experimental UCG using an ex-situ UCG reactor

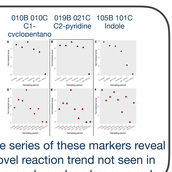
GC-MS

1- Multivariate analysis



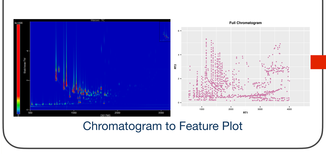
2- Markers discovery



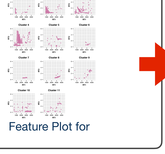


GCxGC-MS

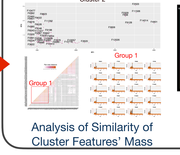
1- Fisher Ratio Selection of Features

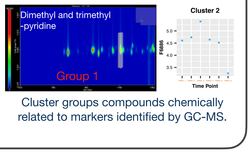


2- Affinity Propagation (AP) Clustering



3- Cluster Analysis





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