

## Non-target screening workflow of plant metabolomes to reveal changes caused by an inflammatory drug incubation

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### Gold statement

- Target, suspect, and non-target analysis workflow to investigate the metabolic profile of *Lemna minor* using a serial coupling HILIC-RPLC-QTOF-MS.
- The workflow bases on the polarity differences of metabolites in HILIC and RPLC columns.
- Revealing the changes in *Lemna minor* metabolomics due to incubation with diclofenac.

### Introduction

Metabolomics approaches can be targeted, suspected, or non-targeted. The target and suspected metabolomics analysis strategy deal with the measurement of a group of metabolites to investigate specific metabolic pathways or to validate biomarkers identified formerly using non-targeted metabolic profiling. In contrast, non-targeted metabolomics approaches involve global profiling of the metabolome. This approach is typically employed in biomarker discovery studies, where comprehensive metabolite identification is generally not the goal. Thus, a non-targeted metabolomics analysis strategy often provides more information than targeted metabolomics, but targeted metabolomics typically is quantitative [1].

### Body

The metabolic profile of *Lemna minor* “known as duckweed” is investigated using suspected and non-target screening analysis workflow. *Lemna minor* belongs to the family Lemnaceae, which is used as an experimental body due to its higher production rate. *L. minor* was incubated for 96 hours with 10 and 100  $\mu$ M diclofenac. After that, *L. minor* was harvested and extracted with three different solvents 100%MeOH, 50%MeOH:50%water, and 100% water, respectively. The residues of the extracts were analysed with serial coupling of HILIC and RPLC column coupled to quadrupole time-of-flight mass spectrometer (QTOF-MS) (AB SCIEX triple TOF 4600, Darmstadt, Germany). The robustness and reproducibility of the coupling were achieved by comparing a mixture of standards that regularly was injected with the sample batches and compared during the experimental time. The workflow considers the suspects and non-target strategies in plant metabolomics. For both strategies: the peaks were picked according to the definite parameters, which were defined using the internal standards. For suspect analysis, the obtained peaks were uploaded into the FOR-IDENT data analysis platform using the compound database PLANT-IDENT (<https://water.for-ident.org/#!home>) to characterize and identify suspects and unknowns. While in non-target analysis, the obtained peaks were evaluated using chemometrics. The main purpose of the statistical data evaluation is to find the relationship between the different and categorize samples. Also, the specific biomarkers, which responsible for the sample differentiation. To achieve this, the unsupervised or supervised classification tools such as Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA), as well as supervised classification techniques, including Partial Least Square Discriminant Analysis (PLS-DA), Orthogonal Partial Least Square-Discriminant Analysis (OPLS-DA) [2].

### Conclusion

The challenge in non-target spectrometric data analysis is to annotate and evaluate the massive generated data. Hence, the performed workflow-enabled identification several of *L. minor* metabolites. Also, it was capable of differentiating and categorize the samples of *L. minor* incubated with or without diclofenac using Chemometrics. Besides, the biomarkers were identified using the PLANT-IDENT database. The workflow gives insights into the metabolomics and chemometrics approaches for examples of plants applied in constructed wetland.

### References

- [1] R.Wahman, *JPBA*. **188** (2020) 113362.

[2] N.P. Kalogiouri, *Anal. Chim. Acta.* **In press** (2020).