Fast detection of pesticides and drugs removed from waste water by plants using Flow Injection Analysis Magnetic Resonance Mass Spectrometry (MRMS)

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Introduction

Treatment of waste water is important to remove pollutants, drugs and other potentially environmentally toxic molecules before release into the nature. Constructed wetlands in France are used to treat water from small villages. Here we present the profiling of plants to understand if pesticides and drugs can be removed from waste water through an accumulation in plants or degradation by environmental factors.

Methods

Data acquisition:
- scimaX MRMS with 7 T superconducting magnet and new dynamically harmonized analyzer cell and 2 omega detection
- mass range m/z 107 – 3000
- ionization: ESI(+) and ESI(-)
- resolving power of 1,350,000 at m/z 200 using magnitude mode
- 28 single scans were averaged for the final mass spectrum

Mass calibration:
- external calibration with NaTFA cluster
- lock mass calibration with compound $C_{12}H_{18}F_{12}N_3O_6P_3$ (potassium adduct in positive ion mode and chlorine adduct in negative ion mode)

Sample Introduction:
- FIA with a sample loop of 20 µL using UPLC Elute HT. During the FIA experiment the flow was reduced to get constant signal for roughly 1.5 min (Fig. 1).

Data processing:
- MetaboScape 4.0Samples:

Sample:

- 8 technical replicates of ZRV (polluted) samples (perfect rejection area); 8 technical replicates of CTRL (control) samples; 2 QC samples (quality control – ZRV and CTRL 1:1 mixed); Methanolic plant extract (stock solutions) were diluted 1:1000 in MeOH for FIA-MRMS.

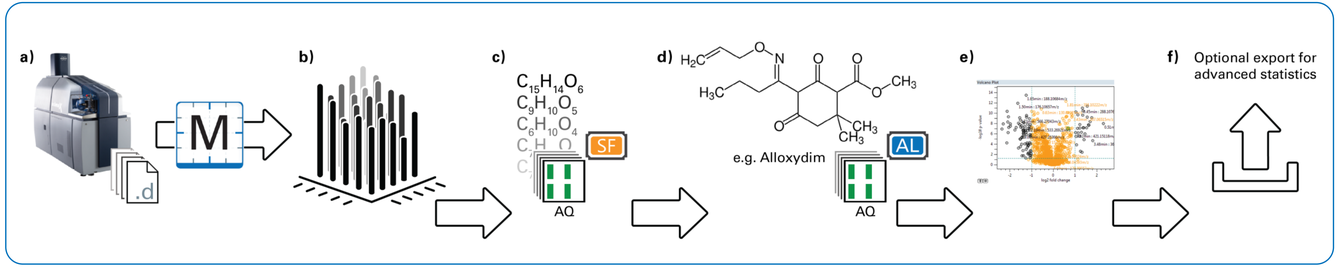

Fig. 1: Schematic MRMS aXelerate workflow: a) FIA-MRMS acquisition using a scimaX MRMS, b) Data processing and evaluation using T-ReX 2D in MetaboScape 4.0, c) Generate list of molecular formula annotations including annotation qualities, d) Putative compound annotations using AnalyteList of known and expected compounds, e) Statistical analysis to identify features of interest, f) Optional export for advanced statistical analyses

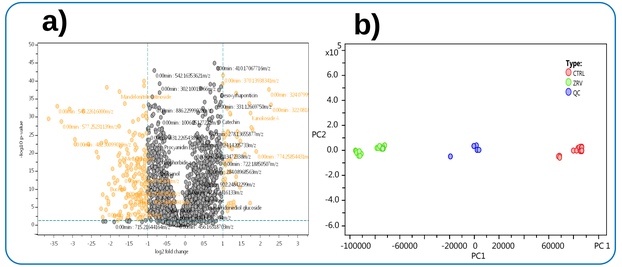

Fig. 2: Statistic analysis of the plant extract samples. a) T-test results (without QC samples) and b) Principal component analysis (PCA) (red: CTRL, green: ZRV and blue: QC samples)

Results

Two poplars (Populus nigra) were planted - one close to the border of a pond (polluted, ZRV) and the other away from the pond (control, CTRL). Mature leaves of these two plants were collected and extracted. Each sample was analyzed in 8 technical and 3 measurement replicates. The data of the ESI(+) and ESI(-) were combined for feature analysis. More than 3,400 features have been found for the plant extract samples (Table 1). Roughly 90% of the detected features could be assigned with a molecular formula using a mass tolerance of only 0.5 ppm. More than 360 compounds have been annotated in the samples using a food data base with nearly 16,000 entries using same mass tolerance of 0.5 ppm. The workflow in shown in Figure 1. Features responsible to differentiate treated and non-treated plant sample were detected by T-test and Principle Component Analysis (PCA) (Figure 2). The samples ZRV and CTRL are well separated in the PCA scoring plot and the QC sample is the center of the plot between both groups. These compounds were analyzed in detail as possible drugs and pesticides. Based on these results 4 pesticides and 4 drug candidates were found by screening versus pesticide and drug data bases (Figure 3a and 3b). The detection of these compounds was based on very accurate mass measurements and the fact that these compounds were only found in the ZRV sample and not in the control sample.

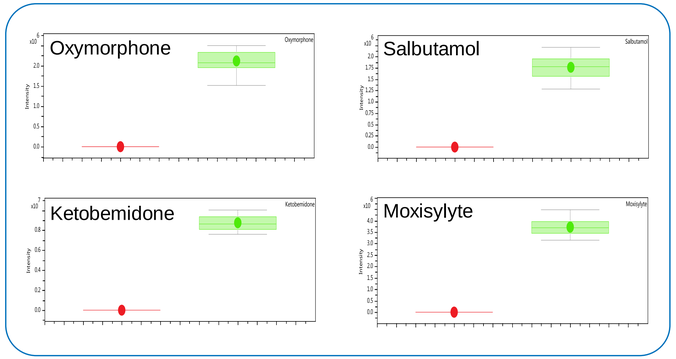

Fig. 3a: Bucket statistic (box plots) of detected drugs in polluted poplar samples (ZRV, green) and control poplar samples (CTRL, red) all detected in positive ion mode.

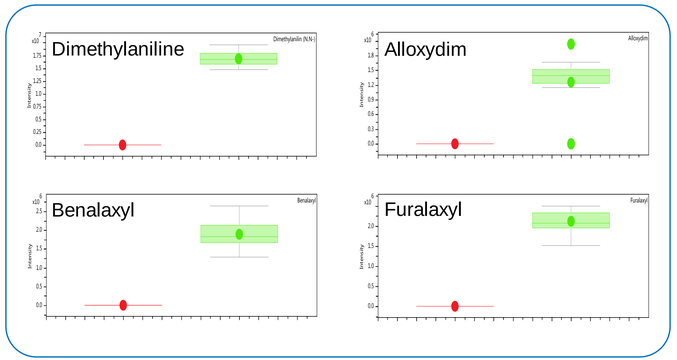

Fig. 3a: Bucket statistic (box plots) of detected pesticides in polluted poplar samples (ZRV, green) and control poplar samples (CTRL, red) all detected in positive ion mode.

Table 1: Detected features, analytes via Food data base and molecular formula calculation of the plant extract samples using ESI(+), ESI(-) and merged data of both polarities.

Measurement	Features	Analytes with Food DB	Mol. Formula with SF calc.
ESI(+)	2,093	100	1,876
ESI(-)	1,444	306	1,404
ESI(+) and ESI(-) merged	3,452	383	3,116

Conclusions

- Pesticides, drugs and their metabolites can be detected by FIA-MRMS in reduced time in plant extracts.
- Low abundant pollutants could be detected from crude extracts without purification.
- FIA-MRMS can be used to understand if pesticides and drugs can be removed from waste water.

MRMS Metabolomics