Strategies for Suspect and Non-target Screening of New Emerging Chemicals in Human Biomonitoring

GC/LC-HRMS Measurement Techniques of Human Matrices and Data Mining of Mass Spectrometry Data for High Throughput Analysis

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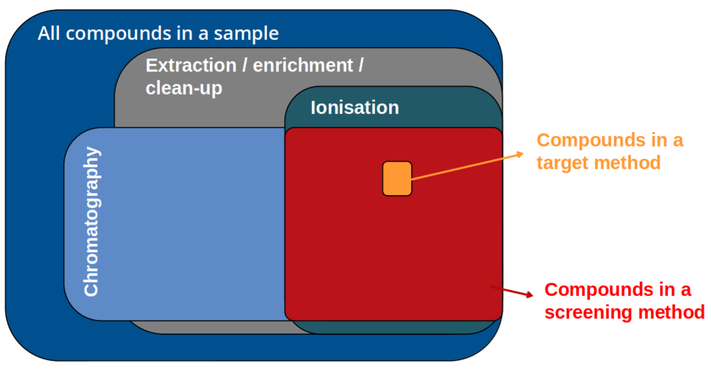
Introduction



Humans are exposed to a large number of chemicals from consumer products, food and through environmental media. To assess this exposure, human biomonitoring (HBM) analyses chemicals in human fluids and tissues. Typically, only a small number of chemicals are covered in targeted methods.

Figure 1: Compound domain covered by screening methods.

Suspect screening- Searching in full scan mass chromatograms for a list of molecular structures of compounds expected in the sample without using a reference standard, with a subsequent tentative identification of suspect hits.

Non-target screening (NTS)- Screening in full scan mass chromatograms for masses of interest based on criteria such as signal intensity or frequency of occurrence and subsequent identification using mass spectrometric information and eventually meta information.

For both strategies, final unambiguous identification of masses is done by comparison to reference standards (targeted analysis).

Main Objectives

The overall aim of this PhD project is the development of suspect and non-targeted screening methods for the detection of a wide range of suspects in human matrices based on liquid and gas chromatography coupled to high resolution mass spectrometry (GC- or LC-HRMS) and to apply these methods for the assessment of human exposure in cohorts studies within the work package 16 of the project **Human Biomonitoring for Europe HBM4EU** (www.hbm4eu.eu). Current projects:

1. Suspect screening methodology on a predefined list of emerging contaminants (xenobiotic amines) on a sample set of a human matrix (urine) using state of the art open source high performance computational tools for prediction and verification.

2. Method development of a non-targeted workflow to compare *in vitro* formed metabolites of pesticides with the occurrence in human samples.

Challenges and Limitations

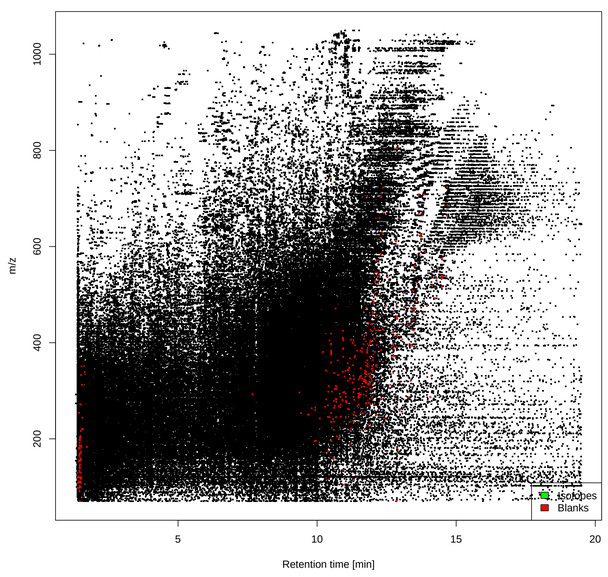


Figure 2: Mass (m/z) vs retention time (min) of all aligned peaks of 40 direct injected urine samples in positive mode. Blank corrected peaks are marked in red. The data set consists of $>$ 180 000 peaks.

- Xenobiotic compounds are typically small peaks against the biological background.
- The substance of interest is typically metabolised resulting in a number of phase I and phase II metabolites.
- While targeted methods rely on the availability of a reference standard, screening techniques rely on prediction techniques to narrow down the list of tentative candidates.
- Screening techniques cannot provide quantitative results but can deliver qualitative results. This results in a list of possible biomarkers of exposure for further targeted analysis.
- HBM relies on large sample sets to account for individual variation. This results in large datasets for subsequent processing.

Comprehensive Workflow Strategies for Suspect Screening

A sample set of 155 human urine samples had been analysed via LC-HRMS. The achieved dataset of fullscan masses had been processed by an automated workflow to generate a list of potential hits. These hits are further used in MS^2 experiments for furter verification. The gained information (MS^2 spectra) was then used for increasing the identification confidence [4] by mass spectral library search, as well as comparing the precursor mass and fragments by formula matching [2] and generating matching scores by comparative open-source *in-silico* fragmentation software [1, 3]. For verification of the established result, representative resulting suspects are further analysed in a targeted approach.

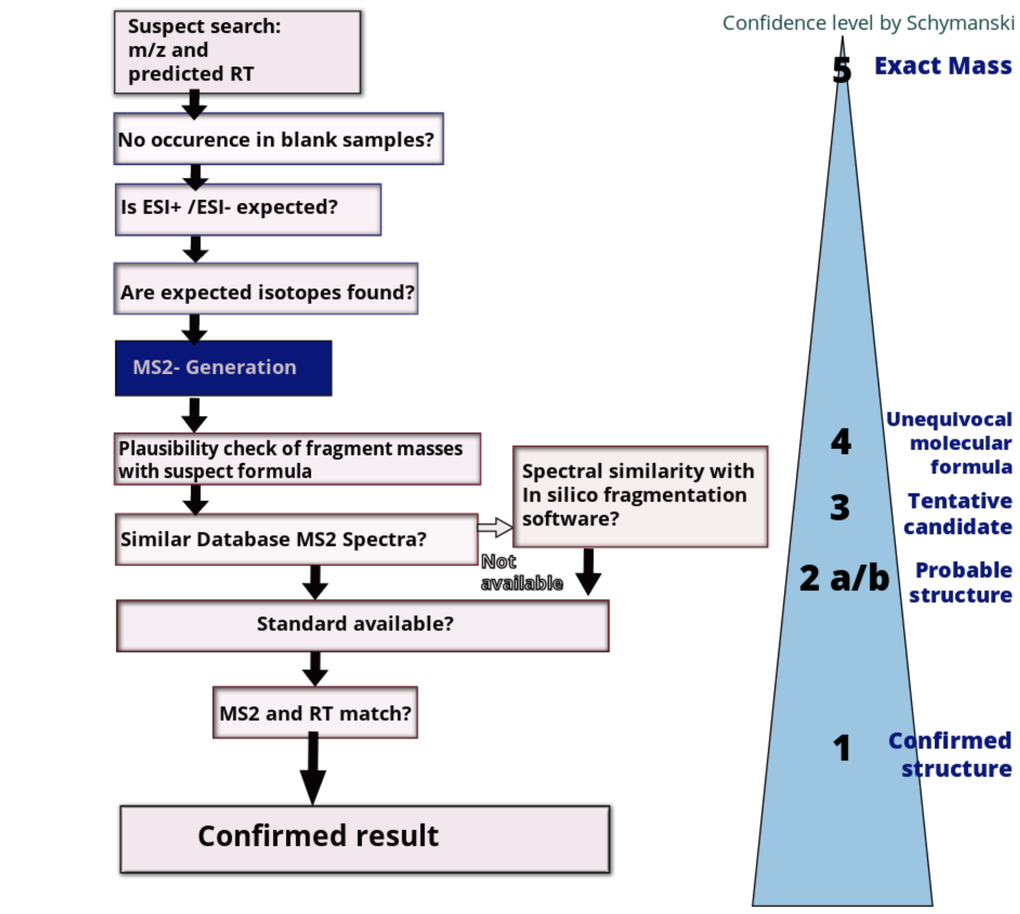


Figure 3: Workflow steps used for screening and identification of metabolites resulting in a filtering of the candidate database into results of different confidence levels proposed by [4].

Non-targeted Metabolomics for Exploring New Biomarkers of Exposure to Pesticides

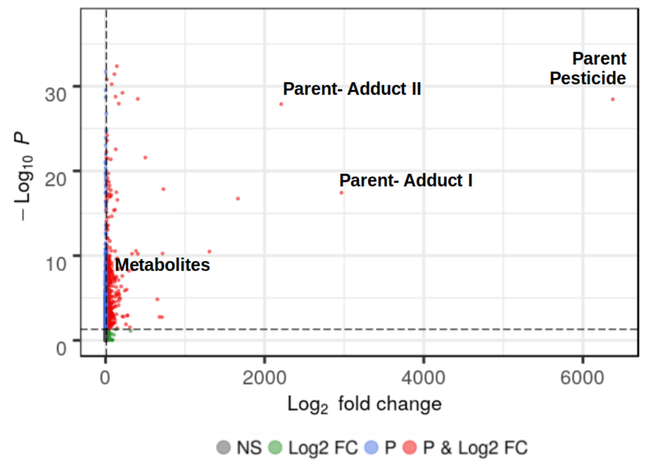


Figure 4: Exemplary volcano plot for the pesticide bendiocarb. Statistically significant peaks resulting from the pesticide exposure are then used for a comparison to human urine samples.

Features of interest for LC-HRMS spectra mining are generated by two complementary approaches:
(1) Mammal metabolites found in literature (registration dossiers).
(2) Experimental *in vitro* exposure of pesticides of interest to pooled human liver S9. Possible metabolites are determined by comparison to other experiments and a negative control (ANOVA).
The statistically significant candidate peaks can then be be automatically scanned by retrospective analysis in a dataset of human urine samples.

Acknowledgements

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