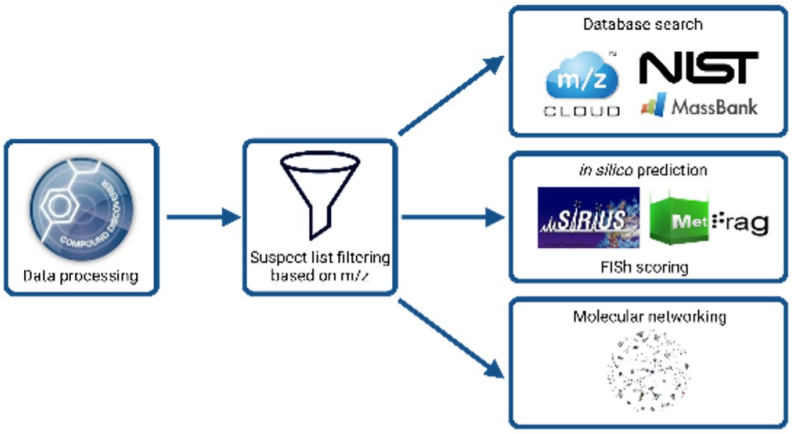
Novel Identification of Human Pharmaceutical Metabolites in Untreated Wastewater by Wide Scope Suspect Screening

C. Meyer[1,2], J. Hollender[1,2]

[1]Environmental Chemistry, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, 8600 Dübendorf, Switzerland, [2]Institute of Biogeochemistry and Pollutant Dynamics, ETH Zurich, Rämistrasse 101, 8092 Zurich, Switzerland

Humans are exposed to a multitude of chemical compounds, including pharmaceuticals. Upon intake, they are often metabolized in order to increase polarity and facilitate their excretion. Detection of these metabolites in the influent of wastewater treatment plants (WWTPs) can give information on the exposure of a large fraction of the human population. Many human metabolites arrive at WWTPs in higher amounts compared to their parents, still many studies analyze the parent compounds or focus on already identified metabolites of single compounds. Therefore, this study aims to identify and analyze metabolites originating from a broad range of pharmaceutical parent compounds by online-SPE-LC-ESI-HRMS/MS in combination with suspect screening. For this purpose, untreated wastewater of three Swiss WWTPs was sampled for one week in early 2022 during dry weather conditions. The suspect list was generated based on the yearly consumption amounts of parent pharmaceutical compounds in Switzerland. To complete the suspect list with the respective human metabolites, literature search, mainly with Drugbank and Swiss medical compendium, was performed. The resulting list encompasses around 1100 metabolites of which 80 were covered by a targeted approach. Feature detection and suspect list filtering were performed with Compound Discoverer. To elucidate the structures of the prioritized features, a combination of database search with mzCloud, MassBank and NIST as well as *in silico* prediction with MetFrag, SIRIUS/CSI:FingerID and FISh scoring was applied. Additional confidence was gained by molecular networking, employing the fact that structural similarity between parent and metabolite can lead to similarity in MS2 spectra. Overall, more than 50 metabolites with confidence levels 2 or 3 could be identified in wastewater, several for the first time. Analytical reference standards were purchased for confirmation.



Applied suspect screening workflow using database search, *in silico* prediction tools and molecular networking for human metabolite identification.