Isomer-Resolved Metabolomic Analysis of Biological Fluids from Drug-Impaired Drivers Using Multimodal Mass Spectrometry with Radical-Driven Ionization and Fragmentation Techniques

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Exogenous compounds such as drugs, pollutants, bacterial toxins and related metabolites exert various effects on biological systems, leading to alterations of endogenous metabolic pathways. Over 70% of xenobiotics are metabolized in the liver, where excess and prolonged exposure can lead to severe organ damage and disrupt its predominant role in lipid metabolism. Untargeted liquid chromatography electrospray ionization mass spectrometry collision-induced dissociation (LC-ESI-MS-CID) techniques are used to evaluate xenobiotic-induced metabolic alterations but are challenged by the identification (ID) of isomeric molecules. Confidence in analyte ID can be increased through orthogonal separation, ionization and fragmentation techniques, such as supercritical fluid chromatography (SFC), electron ionization (EI), atmospheric pressure photoionization (APPI), or electron-activated dissociation (EAD). While EI generates structural informative fragmentation spectra it is restricted to gas chromatography and thereby limited to small thermostable molecules. Contrary to that, ESI and APPI are soft-ionization techniques that can be combined with liquid chromatography and are suitable for a greater variety of compounds. Compared to ESI, APPI does allow radical cation formation with CID spectra usable for EI library searches with over 300,000 molecules and is superior for nonpolar analytes [1]. The ability to fine-tune collision energies enhances the signal of ions related to structural features, such as methyl and double bond positions, and enables access to structural details absent in EI and $[M+H]^+$ CID spectra. This approach has proven particularly useful for the structural elucidation of lipid molecules, where the CID fragmentation of electron-deficient precursors (EDP-CID), such as $M^{+\cdot}$ and $[M-H]^+$, can be used for rule-based de-novo annotation of double bonds, and automated using the MsRadaR R-package [2,3].

This work highlights the development of two radical-driven techniques, dAPPI-EDP-CID and ESI-EAD, and demonstrates their potential in conjunction with SFC and untargeted workflows for the comprehensive screening of xenobiotics, metabolites and lipids in biological fluids from drug-impaired drivers. Preliminary results indicate, that the isomer-resolving potential of ESI-EAD and dAPPI-EDP-CID improves the identification of putative biomarkers linked to drug abuse and enables more precise stratification of subjects based on their drug use behavior.

References:

[1] P. Mueller, R. Bonner, and G. Hopfgartner, Anal Chem, 2022. 94(35): p. 12103-12110.
[2] P. Mueller, G. Hopfgartner, ChemRxiv, 2024.
[3] P. Mueller, G. Hopfgartner, GitHub, 2024, https://github.com/lsmsgeneva/MsRadaR