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Perspectives for DNA adductomics in large-scale exposomics: upscaling sample preparation and preprocessing data

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Study of DNA adduct formation can be highly informative since DNA adducts are markers of exposure to chemical stressors as well as markers of effect, indicating increased risk. Complimentary to targeted DNA adduct analysis, untargeted DNA adductomics can be used for the detection of both known and unknown DNA adducts. DNA adductomics is a relatively young field of research however and methodological improvements can still be made. Therefore, the first objective of the current work was to evaluate thermal acidic vs. enzymatic DNA hydrolysis, followed by DNA adduct purification and enrichment using solid-phase extraction (SPE) or fraction collection; and hydrophilic interaction (HILIC) vs. reversed phase liquid chromatography (RPLC) coupled to high resolution mass spectrometry (HRMS) for DNA adductome mapping and modelling (i.e. assessed using Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA)). At the time, we also lack a clear view on how to preprocess untargeted data. Preprocessing is in fact seldomly applied, apart from signal correction for DNA concentration per sample and the use of a limited number of internal standards. Nevertheless, to obtain high-quality data and improve biological interpretability, proper data preprocessing is an absolute requirement. The second objective of this work was thus to investigate the use of QC (quality control) compared to iQC (internal QC) and QC-based robust locally estimated scatterplot smoothing (LOESS) signal correction in the data preprocessing workflow (based on i.a. superior clustering of QCs, and percentage of explained variance by principal components 1 and 2 in Principal Component Analysis (PCA) plots).

OPLS-DA demonstrated that HILIC compared to RPLC allowed better modeling of the tentative DNA adductome, particularly in combination with thermal acidic hydrolysis and SPE; resulting in more valid models, with an average cumulative Q2(Y) and cumulative R2(Y) of 0.930 and 0.998, respectively. Important to note is that thermal hydrolysis excels in simplicity, cost and time efficiency compared to enzymatic hydrolysis, and can accomodate high-throughput DNA adductomics.

Thermal acidic hydrolysis-SPE-HILIC-HRMS therefore qualifies as the most promising starting point for DNA adductome mapping and modelling in large-scale exposomics. Regarding data preprocessing, QC normalization outperformed iQC and LOESS, and may therefore be put forward as the default data normalization strategy.

Keywords:
Sample extraction; Liquid chromatography; DNA adductome mapping; Data normalization; Upscaling.