Response to Editor

Thanks for editor' constructive comments and suggestions. We have carefully revised our manuscript. To enhance clarity, we present the comments in regular black font, while our responses are displayed in blue normal font. The modified content in both the manuscript is highlighted in red font.

Editor decision: Publish subject to minor revisions (review by editor)

Public justification (visible to the public if the article is accepted and published):

The method and the analysis presented in this manuscript are novel, and go beyond a measurement report. So I support consideration of this manuscript as a research article. However, both reviewers raised the point about representativeness of ESI-MS analysis. In the response, the authors acknowledge these biases, but I think the discussion of these biases are too superficial. I suggest a more critical investigation of each one of the points made (SEC MW characterization being qualitative; bias towards ionizable molecules; fragmentation of larger molecules) and discuss for each one how likely that is the case, and how would it bias the results. For example, there has been some investigation of using ESI for O/C and H/C ratio calculations and they compare well to AMS measurements, so perhaps less likely.

[Response]: Thanks for the editor's insightful comments. We have addressed the viewpoints that were not thoroughly discussed in the initial response.

Regarding the qualitative nature of SEC MW characterization, we have provided a detailed discussion on how the conclusion was reached and the underlying reasons. We point out that the molecular weight calculated through HPSEC is relative and not true due to differences in molecular density, structural similarity, and potential secondary intrinsic reactions between the calibration substances PEG and HULIS. This perspective, supported by Chen et al. (2016) and Ignatev and Tuhkanen (2019), positions the results as qualitative or semi-quantitative.

Second, we have clarified the bias towards ionizable molecules in negative ESI sources. The ESI negative ion mode tends to detect specific compounds based on their inherent characteristics, ionizing functional groups within the molecule that are prone to losing protons, such as carboxylic groups, aldehydes, and hydroxyl groups (He et al., 2023; Song et al., 2022; Lin et al., 2018).

It is important to note that the ESI technique allows for the analysis of the overall molecular composition of HULIS. However, additional research methods are essential for investigating reaction mechanisms and formation processes. We have highlighted this point before introducing the method. Additionally, we have underscored the unique advantages of ESI ion sources, such as disrupting intermolecular forces like hydrogen bonds and van der Waals forces, which is valuable for detecting macromolecular complex compounds. Nevertheless, we acknowledge the need for more extensive research in this area.

Given AMS measurements, it is more likely to involve the strong fragmentation of individual compounds, which differs from the detection principles of HRMS. Consequently, their results are not directly comparable. However, this method can offer a suitable technique to delve deeper into the differences in molecular composition between HMW and LMW HULIS.

In our ongoing work, we plan to employ different technical approaches to compare HULIS obtained under identical conditions, aiming to achieve a more comprehensive understanding. We appreciate the opportunity to address these concerns and enhance the clarity and depth of our manuscript.

[Revise]: The relative statements have been incorporated into the manuscript, specifically in lines 215-219, 238-240, 262-264, 398-404, 410-413, and 424-427.

"It's important to note that the negative ESI source is more sensitive to detecting polar acid compounds, and the reported specific chemical composition here represents a fraction that is biasedly ionized in the negative ESI source, not the entire HULIS composition (He et al., 2023; Song et al., 2022)."

"Due to the potential differences in molecular densities between the calibration standards (PEG) and BB WSOC, the reported molecular weight of HULIS calculated by HPSEC herein is only estimate."

"Note that these values are estimates rather than absolute, given the absence of the appropriate aerosol HULIS standards (Fan et al., 2023; Wong et al., 2017)."

"It is worth noting that the ESI- HRMS could reveal molecular composition of a subset of organic molecules that are biased ionized in the negative ESI source, particularly acid compounds such as carboxylic and sulfonic acids, and may not represent the entire HULIS composition (He et al., 2023; Lin et al., 2012). Additionally, HRMS techniques are often known to be biased towards low masses below 600 Da (Lin et al., 2012; Wang et al., 2019), suggesting that the molecular sizes calculated by HRMS are likely underestimated."

"The similarity in low m/z range among HMW and LMW HULIS and other bulk HULIS may indicate their shared fundamental molecules, further suggesting the potential disassembly of larger molecules."

"Moreover, SOA-derived HULIS generally contain more polar molecules (e.g., -OH, -COOH) (Di Lorenzo et al., 2017; Fan et al., 2020; Huo et al., 2021), making them more susceptible to deprotonation and leading to the generation of a greater number formulas under negative ESI conditions."

[Reference]:

(1) Chen, M., Kim, S., Park, J.-E., Jung, H.-J., Hur, J., 2016. Structural and compositional changes of dissolved organic matter upon solid-phase extraction tracked by multiple analytical tools. Analytical and Bioanalytical Chemistry 408, 6249-6258.

(2) He, T., Wu, Y., Wang, D., Cai, J., Song, J., Yu, Z., Zeng, X., Peng, P.a., 2023.

Molecular compositions and optical properties of water-soluble brown carbon during the autumn and winter in Guangzhou, China. Atmospheric Environment 296, 119573.

(3) Ignatev, A., Tuhkanen, T., 2019. Step-by-step analysis of drinking water treatment trains using size-exclusion chromatography to fingerprint and track protein-like and humic/fulvic-like fractions of dissolved organic matter. Environmental Science: Water Research & Technology 5, 1568-1581.

(4) Lin, P., Fleming, L.T., Nizkorodov, S.A., Laskin, J., Laskin, A., 2018. Comprehensive Molecular Characterization of Atmospheric Brown Carbon by High Resolution Mass Spectrometry with Electrospray and Atmospheric Pressure Photoionization. Analytical Chemistry 90, 12493-12502.

(5) Song, J., Li, M., Zou, C., Cao, T., Fan, X., Jiang, B., Yu, Z., Jia, W., Peng, P.a., 2022. Molecular Characterization of Nitrogen-Containing Compounds in Humic-like Substances Emitted from Biomass Burning and Coal Combustion. Environ. Sci. Technol. 56, 119-130.