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Viewpoint

Advancing Critical Applications of High Resolution Mass Spectrometry for DOM Assessments: Re-Engaging with Mass Spectral Principles, Limitations, and Data Analysis

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The development of high resolution mass spectrometry (HRMS) has been immensely valuable to the environmental chemistry community. HRMS has been used to identify organic compounds in environmental matrices[1] and to characterize complex mixtures of organic compounds present in dissolved organic matter (DOM).[2,3] In this work, we refer to "compounds" as the elemental compositions determined from the exact masses detected by HRMS. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) using a high magnetic field has opened the door for unparalleled mass resolving power and mass accuracy necessary for nontarget, molecular-level identification of organic compounds in atmospheric, aquatic (freshwater and marine), and terrestrial DOM mixtures, in both carbon-poor and carbon-rich environments.

The application of HRMS in the identification of organic compounds from complex mixtures such as DOM has surged over recent decades. Initial publications focused on identifying molecular formula of DOM compounds and characterizing substances in environmental isolates.[2,3] Quite rapidly, studies employing HRMS shifted to assessing DOM compound properties, identifying changes in molecular formula to specific biological and chemical processes (e.g., microbial biodegradation, natural photochemical oxidation). However, sample preparation and HRMS biases can confound results and subsequent interpretations. For example, sample preparation procedures on DOM samples prior to HRMS (e.g., XAD isolation, solid phase extraction, reverse osmosis, ultrafiltration), can bias the results to the fraction recovered. HRMS instruments (e.g., FT-ICR MS, Orbitrap) can introduce bias, for example, relative ionization efficiencies versus sample type.[4] Another common example of a potential issue with HRMS for DOM analysis is the focus on mass spectral peak intensity correlations with peak intensity data from non-HRMS-type analytical instruments targeting other various DOM properties, e.g., fluorescence. Given that HRMS peak intensities are a product of sample, ionization efficiency, and instrument setting biases, correlations without bias context or further analyses cannot describe absolute linkages. Lastly, HRMS detects ions of lower molecular weights (<1000 Da) and therefore represent ionizable subsets of the total DOM pool.

The wealth of HRMS data offers many opportunities to conduct diverse analyses, where new correlations may drive forward knowledge on DOM. Recognizing the inherent biases and employing caution is recommended, as such biases and technical choices can lead to different perspectives on the chemical understanding of the material.[4] These limitations warrant discussion when interpreting HRMS results for DOM assessments and connection to physical or biogeochemical processes, or the role of DOM in ecosystem function. In many cases, HRMS data has been used to make inferences about DOM properties in conjunction with complementary data, but these possible correlations (e.g., using peak intensities) often lack proper causal evaluation and/or discussion of bias (e.g., ionization efficiencies).

As we move forward with DOM HRMS applications for identification and characterization purposes, targeted ap-

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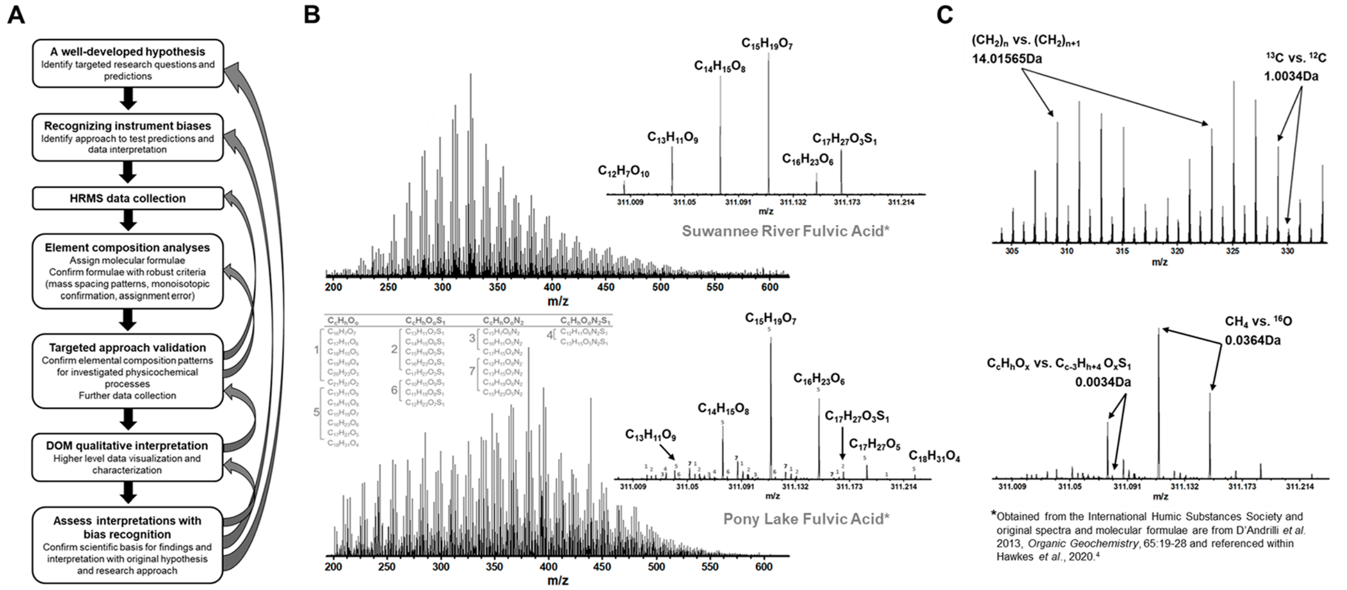

*Obtained from the International Humic Substances Society and original spectra and molecular formulae are from D'Andrilli *et al.* 2013, *Organic Geochemistry*, 65:19-28 and referenced within Hawkes *et al.*, 2020.[4]

Figure 1. High resolution mass spectrometry (HRMS) dissolved organic matter (DOM) assessment steps showing (A) a robust workflow, (B) mass spectra for Suwannee River (top) and Pony Lake (bottom) fulvic acids with their molecular formula at m/z 311 (insets),[4] and (C) mass spacings for formula assignment (Dalton; Da) and targeted research approaches.

proaches will advance this field of research. We propose that the use of HRMS is approached with a well-defined hypothesis (Figure 1). If a study investigates biological, photochemical, and/or other ecosystem processes of DOM production and transformation, an intent to collect specific causal molecular information from HRMS will improve mechanistic understanding. For example, DOM photooxidation creates identifiable shifts in molecular weights, functional groups, and in oxygen and heteroatom content.[5] Collecting and interpreting HRMS data with clear expectations of chemical indicators will avoid perfunctory, misidentified, or retroactive correlations. Even though it is possible that a given DOM property statistically correlates with data obtained by HRMS, it may not confirm a linked causal effect. We find that in many cases, there is a limited scientific basis for the proposed correlations and simply having HRMS data does not guarantee that a clear conclusion can be reached. Instead, using an approach that begins with a well-defined hypothesis, has clear research questions, and pursues targeted analysis of the DOM chemistry will produce sound molecular-level interpretations and understanding (Figure 1A).

HRMS researchers begin molecular DOM interpretations directly on the formula information based on individual masses and elemental content (Figure 1B), before visualizing larger composition data on van Krevelen diagrams and performing supplementary analyses. This is an essential step, beginning with the data generated first, prior to higher-level assessments. HRMS researchers rely on the principles governing mass spectra generation and the strength of highly resolved ion peaks in determining unambiguous assignments of molecular formula.[2−5] Without careful analysis of fundamental mass spectral spacing patterns of highly resolved peaks (e.g., confirming monoisotopic peaks having $^{12}C_n$ versus $^{12}C_{n-1}{-}^{13}C_1$ used to confirm singly charged ions; Figure 1C), assigning individual peaks to DOM molecular formula would not be possible within a reasonable error margin (e.g., <1 ppm).[2−5] Thus, there is a reliance on the repeating spectral patterns to determine and confirm DOM composition. Mass spectral spacing patterns continue to be undeniably powerful for DOM molecular-level understanding as no manual assignments or algorithm can produce unambiguous molecular formula without them. The utility of mass spectral spacing patterns, for example, between two peaks (monoisotopic spacings) and among multiple peaks (e.g., methylene homologous series, CH_4 versus ^{16}O)[2,3] are reliable resources to first understand and then analyze data (Figure 1C). We also assert that the utility of mass spectral spacing patterns is not limited to these examples and can continue to evolve to target molecular information on specific processes, for example, photooxidation. Therefore, we propose a refocusing on the utility of mass spectral spacing patterns because they not only set the foundation of confirming charged ions (e.g., singly versus doubly or multiply) and formula assignments (and adducts) for DOM mixtures, but also are essential resources for targeted inquiries and biogeochemical relationships.

Ultimately, HRMS allows DOM researchers to investigate complex organic mixtures. However, a clear understanding of the instrument's principles and limitations is essential, especially when relating HRMS data to other complementary analytical techniques having their own principles and limitations. We assert that with the abundance and breadth of information gained by HRMS combined with its biases and limitations, the most informative use of HRMS data requires the following approach: targeted, hypothesis-driven research, intentional complementary assessments for other physicochemical information, and utilization of spectral patterns that center on molecular chemical responses of individual processes. Using this workflow, spurious correlations with complementary analyses will be reduced.

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Notes

The authors declare no competing financial interest.

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