New Separation Method Reveals Thousands of Hidden Compounds in Renewable Bio-Oils

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Fast pyrolysis of lignocellulosic biomass yields oxygen-rich bio-oils, which serve as promising feedstocks for sustainable fuel and chemical production. However, their molecular complexity and abundance of acidic, polyfunctional species complicate analysis by direct infusion FT-ICR mass spectrometry (MS), which suffers from ion suppression and limited structural resolution. To address this, MagLab users developed a partition-based chromatography method using a polymeric stationary phase with amino functionalities (**Figure 1a**). This phase retains acidic compounds through reversible acid–base interactions. A solvent gradient from methanol to water with diethylamine creates a high-pH, hydrogen-bonding environment that separates compounds based on their acidity and polarity. As more water is added, different types of acidic compounds—like phenols, carboxylic acids, and lignin-based oligomers—elute sequentially for MS analysis.

Coupling the separation to 21T FT-ICR MS (**Figure 1b**) enables assignment of tens of thousands of molecular formulas per experiment. This time-resolved approach captures compositional trends, as shown with van Krevelen diagrams (Figure 1c) plotting formulas by H/C and O/C ratios for selected scans. Early spectra (e.g., scan 75) contain methanol-soluble carbohydrates (high H/C, O/C); intermediate scans (e.g., 150) show biomass-like polyphenols; and later scans (e.g., 190/225) highlight aromatic, acidic species (low H/C). The method also resolves structural isomers: compounds with the same molecular formula but different atomic connectivity. For example, the single-ion chromatogram for $C_{12}H_{20}O_{10}$ reveals two distinct peaks (Figure 1d), which indicates the presence of isomers. One likely corresponds to cellobiosan, a known pyrolysis product; the second may represent a more acidic variant. Such detail is not accessible by direct infusion analysis and is essential for improving bio-oil upgrading processes.

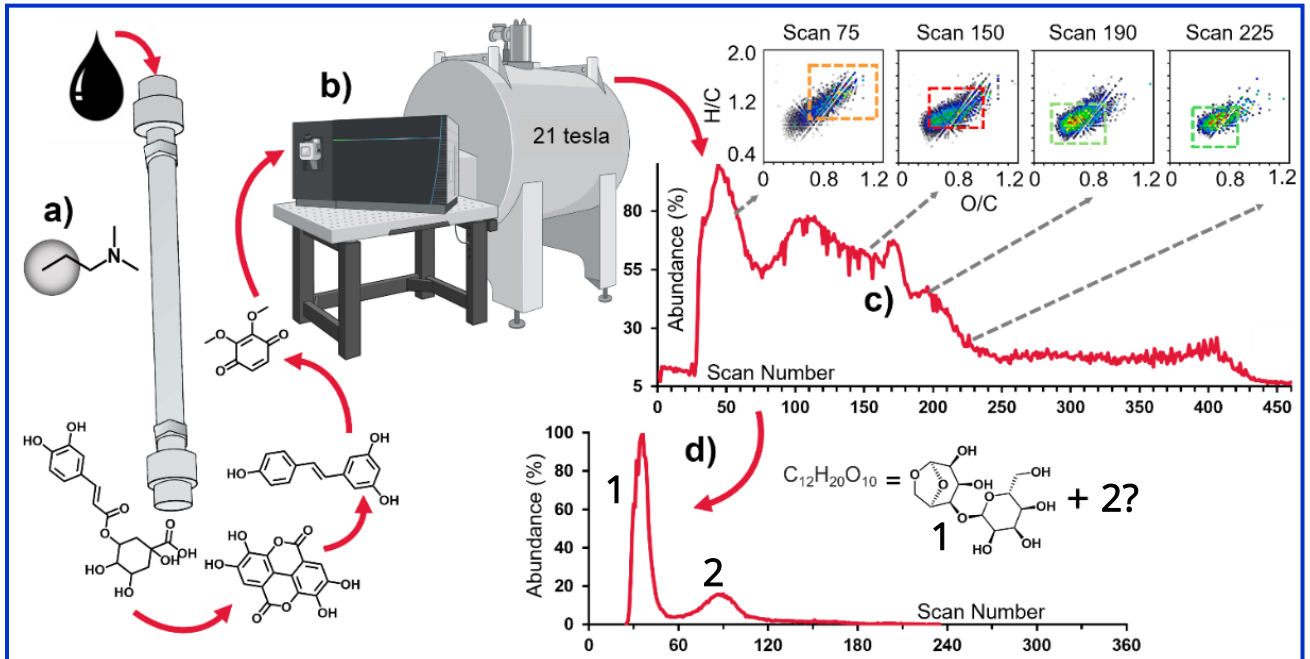

Figure 1. (a) Dimethylaminopropyl stationary phase separates bio-oil components by acidity and polarity. Non-acidic species elute first; polyphenols, carboxylic acids, and polyfunctional compounds elute later. (b) Effluent is analyzed by 21T FT-ICR MS, acquiring one spectrum every ~5 seconds. (c) Total ion chromatogram with selected scans displayed as van Krevelen diagrams (H/C vs O/C), revealing compositional trends from carbohydrates to aromatic acids. (d) Single-ion chromatogram for $C_{12}H_{20}O_{10}$ shows two peaks, indicative of isomers. The first likely matches cellobiosan (structure shown); the second may include acidic groups. The number of unique formulas detected with this chromatographic approach is nearly twice that of direct infusion.

Facilities and instrumentation used: Ion Cyclotron Resonance Facility – 21T FT-ICR MS. **Citation:** Chacón-Patiño, M. L.; Frye-Jones, J. W.; Anderson, L. C.; Robbins, W. K.; Vallverdu, G. S.; Tello-Rodríguez, Á. J.; Ruiz, W.; Gascon, G.; Rüger, C. P.; Dayton, D. C.; Giusti, P.; Mase, C.; Barrière-Mangote, C.; Afonso, C.; Bouyssiere, B.; Rodgers, R. P. Detailed Molecular Composition of Wood Pyrolysis Bio-Oils Revealed by HPLC-FT-ICR MS. Energy Fuels 2025, 39 (7), 3575–3588. https://doi.org/10.1021/acs.energyfuels.4c05674. Work funded by UCGP – 2023.