

## **Title: Liquid chromatography-mass spectrometry analysis of lignin depolymerized products from plant hydrolysates**

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**Project Goals: Establish the scientific knowledge and new technologies to transform the maximum amount of carbon available in bioenergy crops into biofuels and bioproducts.**

There is a growing demand to produce renewable and sustainable energy from plant-based biomass. However, the structural support tissue of vascular plants, i.e. lignin, is recalcitrant under ambient conditions, which poses a considerable technical challenge to the conversion of lignin to fuels and value-added coproducts. Consequently, lignin requires chemical (e.g., base catalysis and ionic liquid, IL) and/or enzymatic pretreatment for effective depolymerization. In this regard, the physicochemical properties of ILs enable the efficient deconstruction and fractionation of biomass needed for conversion of lignocellulosic feedstocks to fuels and chemicals and offers the potential for lignin valorization towards enhanced economic viability of lignocellulosic biorefineries. As a result, it is imperative that the resulting hydrolysates from such refineries be characterized. To this end, we have developed a high-throughput liquid chromatography-mass spectrometry (LC-MS) method to quantify products of lignin depolymerization and/or residual phenolic monomers from hydrolysates produced via cholinium lysinate ([Ch][Lys]) IL pretreatment and enzymatic saccharification. The method showed good linearity ( $R^2 = \geq 0.99$ ) and retention time repeatability (% RSD = < 0.5) for the analytes tested. The method revealed different phenolic profiles for poplar and sorghum hydrolysates, with 4-hydroxybenzoic acid being prominent in both. This suggests that the method can be used to determine the phenolic contents of hydrolysates, assess phenolic compound viability (i.e., extent of degradation) and uptake by microbes in the biorefinery, and potentially quantify the amounts of value-added coproducts produced.

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