

FRACTIONATION OF AN OXIDATIVE LIGNIN DEPOLYMERIZATION MIXTURE USING CENTRIFUGAL PARTITION CHROMATOGRAPHY (CPC)



APPLICATION NOTE AN1045

CPC APPLICATION BENEFITS

- Process an input sample comprising 25 wt% of known and desired compounds without column clogging due to the remaining mass of unknown oligomeric
- Enrich low-abundance compounds
- Global fractionation of a complex biomass reaction extract

ADDRESSED ISSUES

- Valorization of low-value lignin
- Deepen the attainable analytical insights of lignin structure and reactivity
- CPC permitted the efficient recovery of all injected materials, including oligomeric compounds

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INTRODUCTION

Lignin (Figure 1) is an abundant phenylpropanoid polymer found in all plant matter. It has historically been treated as an impurity in the paper pulping and ethanol biorefinery industries where it is burned as a low-value fuel or sold at low prices. However, developing higher value applications of lignin would help biorefineries operate at higher efficiencies. Lignin has been identified as a viable renewable source of aromatic compounds that could replace petroleum due to its high aromatic content. The promise and complexity of lignin's structure has spurred research into efficient depolymerization techniques that yield various classes of aromatic compounds, which could serve as platform chemicals in producing plastics, pharmaceuticals, flavorants, dyes, and many other societally important products. While there are many examples of lignin depolymerization strategies, very little effort has been directed at isolating pure compounds from the typically

complex residues. Solute recovery is severely hindered by the presence of oligomeric materials that often occlude the necessary high-resolution separation techniques, such as flash/ preparative/ simulated moving bed chromatographies or selectively permeable membranes.¹

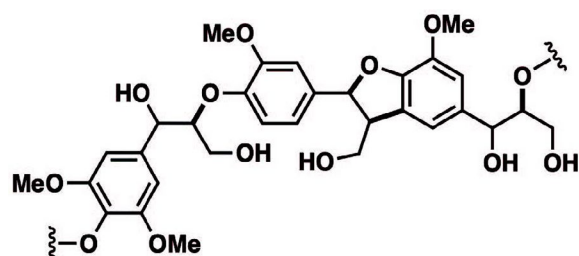


Figure 1
Representative fragment of lignin structure

In this application note, based on work conducted in the lab of Shannon Stahl,² a two-dimensional separation is conducted on an oxidative lignin depolymerization mixture using only a CPC 1000. The liquid stationary phase proves robust, despite the injection of a sample that is ~75 wt% dimeric, trimeric, etc. compounds that would cause most other purification techniques to fail. In addition to the effective recovery of multiple compounds (Figure 2) in good purities, the technique also reveals the value of CPC as a tool for gaining deeper insights into depolymerization mixtures that would otherwise go unnoticed. The breadth of capabilities offered by CPC make it a powerful addition to any research- or commercial-scale biomass facility.

MATERIALS AND METHODS

Oxidative lignin depolymerization residue purification was performed on a CPC 1000 (Figure 3) connected to a PLC 2250 Purification System (Compact LC system) which is configured with a 250 mL/min quaternary gradient pump, automatic injection valve, backflush valve, UV/VIS detector, fraction collector, and Glider Software.

CPC solvent system:

A two-dimensional separation consisting of the Arizona L solvent system (pentane / ethyl acetate / methanol / water) serving as the first dimension followed by a halogenated system (10:6:4 dichloromethane / methanol / water) as the second dimension. Both runs were conducted in the ascending mode with both the lower and upper phases automatically generated online using Glider Software and the PLC 2250 low pressure gradient valves.

Sample preparation:

1.6 grams of CuAHP poplar lignin was subjected to alkaline oxidative depolymerization. The reaction solutions were acidified, extracted with ethyl acetate, and concentrated until a residue remained. The residue was dissolved in 10 mL of Arizona L lower phase and drawn up into a disposable syringe affixed with an 0.45 micron syringe filter. The syringe plunger was depressed to load 8.5 mL into a 20 mL loop on the PLC 2250 and automatically injected onto the CPC 1000.

The second dimension of purification using the halogenated solvent system involved repeating the above procedure with the concentrated residue of the Mixed Fraction peak from the first separation.

CPC separation method:

Both steps of the separation method were programmed into Glider Software to resolve each compound.

The column was first filled in ascending mode with the aqueous mobile phase of the AZ L solvent system at 100 mL/min and 500 rpm. The organic

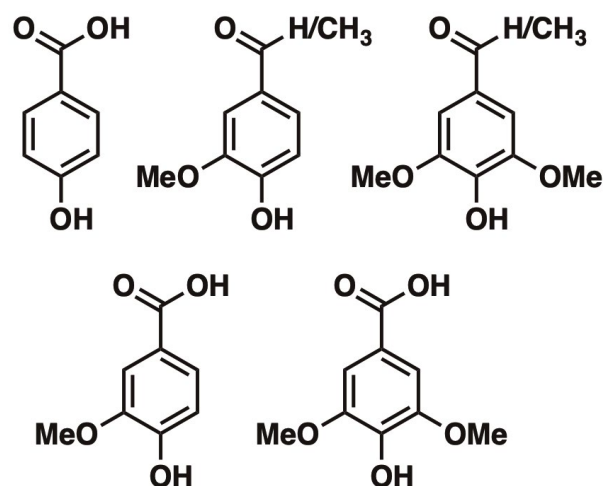


Figure 2

Compounds obtained from oxidative lignin depolymerization



Figure 3

CPC 1000 with a PLC 2250

mobile phase of the solvent system was pumped at 30 mL/min and 1400 rpm.

After injection, elution with mobile phase was performed for the duration of 50 min, then an extrusion step was applied to recover any remaining compounds from the sample in the stationary phase. During this last step, the fresh aqueous phase was pumped at 100 mL/min and 500 rpm.

The second dimension was conducted in the ascending mode as well with completely identical conditions except for 25 mL/min mobile phase flow rate at 1200 RPM for 45 min. Effluent was monitored by UV detection at 290 nm and 310 nm, and in scan mode from 200 to 600 nm. 25 mL fraction volumes were collected for the entire duration. After solvent evaporation, the mass of each residue was determined. HPLC analysis was used to determine the recovery and purity of the desired solutes in the concentrated residues.

RESULTS AND DISCUSSION

CPC separation of the oxidatively depolymerized lignin was conducted in two separate steps as shown in Figure 4. As shown in the CPC chromatogram in Figure 5, the first purification provided four peaks corresponding to vanillin, a mixed fraction of monomers, syringic acid, and oligomeric compounds by order of elution. The mixed fraction of monomers contained phydroxybenzoic acid (pHBA), vanillic acid, and syringaldehyde. These compounds were isolated in a separate CPC run shown in Figure 6. Each of the distinct compounds from both separations had their fractions pooled and evaporated, providing residues of enriched purities for each. The mass of each residue was weighed on an analytical balance while HPLC analysis provided the mass of the desired compounds in each injection and recovered residue. These values were used to compute the recovery and purity of each compound, as summarized in Table 1.

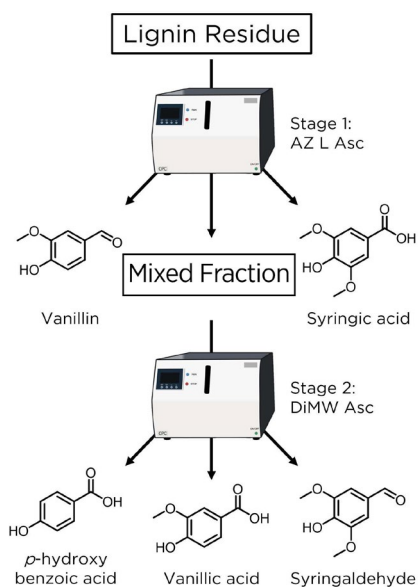


Figure 4
The small molecules in the residue are separated by two sequential CPC runs.

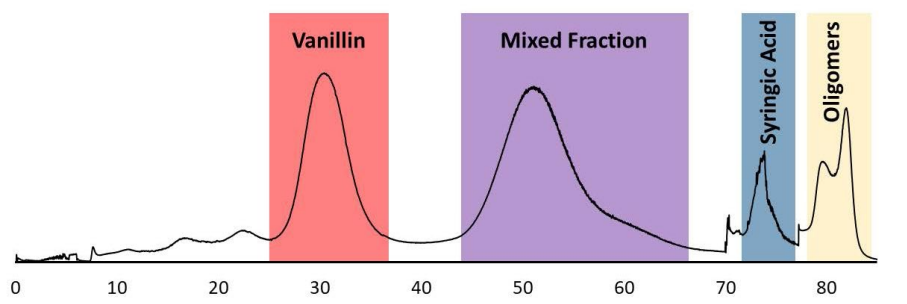


Figure 5
CPC chromatogram of the Arizona L Ascending separation applied to oxidative lignin depolymerization residue. (UV detection at 270 nm)

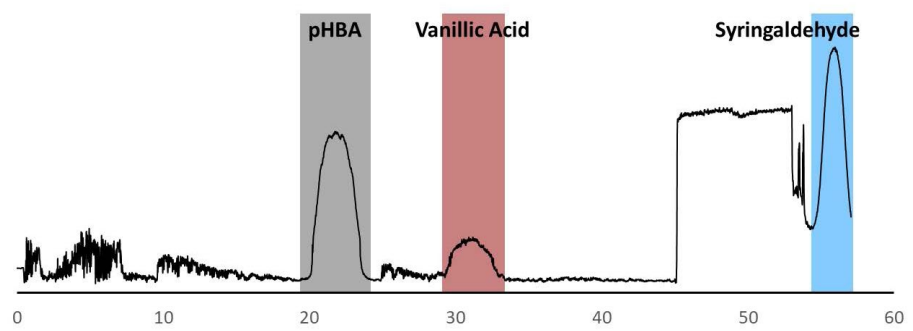


Figure 6
CPC chromatogram of the dichloromethane/methanol/water Ascending separation applied to the mixed fraction. (UV detection at 270 nm)

Table 1

Mass recovered, recovery yield, and purity of each identified compound obtained after two-stage CPC purification.

PARAMETERS	VANILIC ACID	SYRINGIC ACID	VANILLIN/ ACETOVANILLONE	SYRINGALDEHYDE/ ACETOSYRINGONE	PHBA
Mass Recovered (mg)	11.7	22.3	68.4	128.5	37.5
Recovery	86%	79%	91%	91%	82%
Purity	81%	50%	65%	87%	72%

CONCLUSIONS AND BENEFITS

The realization of a biorenewable, lignin-derived platform of chemicals is stuck in a catch-22. The market for lignin-derived compounds, and therefore their perceived economic value, is nonexistent. On the other hand, petroleum resources benefit from the momentum of having a clearly defined, fully implemented refining process and steady demand. Biorenewable materials intended to replace these petroleum resources are ultimately at a disadvantage until economically viable refining processes are identified; especially one that facilitates the effective utilization of all produced materials.

In this study, a two-stage CPC purification sequence helped provide a bridge over the gap between active research and efficiently using lignin by achieving two goals:

- The global fractionation of a complex biomass reaction extract comprising various organic compounds including:
 - › vanillin, a high value flavorant.
 - › p-hydroxybenzoic acid, the core of all parabens.
 - › vanillic acid, syringic acid and syringaldehyde, proposed platform chemicals that exhibit antioxidant properties.
 - › oligomeric fractions, high molecular weight compounds that can be incorporated into future materials. Improved potential for gaining analytical insights.
- Improved potential for gaining analytical insights.

The completely liquid nature of CPC permitted a quick and direct purification of both known and unknown compounds with the potential for commercial use and structural analysis, adding value to complex and intransigent lignin residues, the largest natural source of aromatic materials.

REFERENCES

1. <https://pubs.acs.org/doi/10.1021/acscentsci.1c00729>
2. <https://stahl.chem.wisc.edu/>

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