Supporting Information

From Lignin to Valuable Aromatic Chemicals: Lignin Depolymerization and Monomer Separation via Centrifugal Partition Chromatography

Manar Alherech,† Surajudeen Omolabake,‡ Christopher M. Holland,† Gracielu E. Klinger,‡ Eric L. Hegg,† and Shannon S. Stahl†,*

†Department of Chemistry, University of Wisconsin-Madison, 1101 University Avenue
Madison, Wisconsin, 53706, United States
‡ Wisconsin Energy Institute, University of Wisconsin–Madison, Madison, Wisconsin 53726, United States
§ Department of Biochemistry & Molecular Biology, Michigan State University, 603 Wilson Road, East Lansing, Michigan, 48824, United States

*Corresponding Author: stahl@chem.wisc.edu

Table of Contents:

1. General Experimental Considerations .......................................................... S2
2. Isolation of Cu-AHP lignin ...................................................................... S3
3. General procedure for lignin oxidative alkaline depolymerization ............... S3
4. Procedure for HPLC analysis and calibration curve development .............. S4
5. General procedure for measuring partition coefficients (K_P) .................. S6
6. General procedure for screening Arizona solvents on CPC ...................... S8
7. Varying Arizona solvents and flow direction on CPC .............................. S9
8. Assessment of the 10:6:4 CH_2Cl_2/MeOH/H_2O solvent system ............. S10
9. NMR analysis of the 10:6:4 CH_2Cl_2/MeOH/H_2O solvent system .......... S11
10. Purity and recovery assessment of Arizona L Asc separations ............... S12
11. Purity and recovery assessment of 10:6:4 CH_2Cl_2/MeOH/H_2O Asc separations ... S14
12. NMR spectra of solutes recovered from CPC separations ..................... S16
13. Applying one-step 10:6:4 CH_2Cl_2/CH_3OH/H_2O Asc to lignin depolymerization ... S19
14. Determination of solute enrichment factors ........................................ S20
15. Analysis of the remaining collected fractions in lignin sample separation ... S20
16. References .............................................................................................. S22
1. General Experimental Considerations

Hybrid NE-19 poplar was grown at the University of Wisconsin Arlington Agricultural Research Station and provided to us by the Great Lakes Bioenergy Research Center. All commercial reagents were purchased and used as received. Reagent grade pentane, reagent grade ethyl acetate, reagent grade methanol, reagent grade dichloromethane, 1,4-dimethoxy benzene, sodium hydroxide, vanillin, syringaldehyde, vanillic acid and \( p \)-hydroxybenzoic acid were purchased from Sigma-Aldrich. Syringic acid was purchased from Oakwood Chemical. \( \text{CuSO}_4 \cdot 5 \text{H}_2\text{O} \), HPLC grade methanol, HPLC grade ethyl acetate and HPLC grade pentane were purchased from Fischer.

All lignin depolymerization reactions were prepared in hollowed PTFE vessels which were then arranged in a 1-L, stainless steel Parr vessel. The reactor temperature was controlled by a Parr 4838 Reaction Controller with a heating mantle sleeve and a thermocouple. High Performance Liquid Chromatography analysis were performed through LabSolutions software on a Shimadzu Prominence system equipped with a 150 mm x 4.6 mm ID, 5-micron particle size Restek C18 column heated to 35 °C. Calibration curves and sample traces were acquired with a deuterium lamp equipped Photo-Diode Array monitoring elution at 280 nm ± 0 nm bandwidth. CPC separations were performed using a Gilson PLC 2250 Purification System liquid handler connected to a Gilson CPC 1000. CPC Methods were prepared with Glider Prep, the built-in panel software, with the following general parameters for all runs unless otherwise specified:

**Settings (Tab)**
- Equilibration Mode – Equil. (Table)
- Injection Mode – Valve Inj. + Wait
- Max Pressure – 70 bar
- Stop Mode – Pause
- Automatic Gradient Optimization – Off

**Detection (Tab)**

| CH #1 (Tab) – UV600:SCAN | Time | Scan < | >> Scan | Collect | Threshold | FI |
|--------------------------|------|--------|---------|---------|-----------|----|
|                          | 00 s | 200    | 600     | No      | 300       | 1  |

| CH #2 (Tab) – UV600:SIG1 | Time | WL (nm) | Collect | Threshold | FI |
|--------------------------|------|---------|---------|-----------|----|
|                          | 00 s | 280     | Yes     | -500      | 1  |

**Collection (Tab)**

| Time | Local | Volume | Mode | Action |
|------|-------|--------|------|--------|
| 00 s | No    | Full   | Waste| None   |

**Safety Note** – caution should be used when conducting reactions at temperatures significantly higher than the solvent boiling point. Efforts should be made to maintain an operating pressure well within the safety limits of the reactor vessel, which should be equipped with a fail-safe burst disk. Always use a blast-shield and heat resistant gloves when handling a pressurized, heated vessel.
2. Isolation of Cu-AHP lignin

The following procedure was adapted from previously published work. Milled NE-19 hybrid poplar (1 kg) was incubated at 30 °C in 10 L of 270 mM sodium hydroxide solution for one hour. The liquor was removed (5 L) and the remaining slurry was washed with 10 L deionized water. To the biomass was then added 3.95 L of distilled water and the following aqueous solutions: 500 mL of 5 M sodium hydroxide, 125 mL of 40 mM CuSO₄, and 125 mL solution containing both 40 mM CuSO₄ and 160 mM 2,2'-bipyridine. The combined solution was agitated to ensure homogeneity and incubated at 30 °C. Each hour, 30 mL of 30% H₂O₂ was added for 10 hours. The solution was then allowed to incubate for an additional 14 hours while shaking. At the end of the incubation period, the liquid phase was isolated by filtration and acidified to pH 2.0 with 72% (w/w) sulfuric acid. The resulting precipitate was centrifuged, washed, and resuspended with pH 2.0 aqueous sulfuric acid, then recovered by centrifugation. Finally, the liquid was decanted and the solid was lyophilized to yield a light tan powder.

3. General procedure for lignin oxidative alkaline depolymerization

To a hollowed, 100 mm tall, 26 mm O.D., and 24 mm I.D. PTFE vial were added a 1.5 mm x 7.9 mm PTFE coated stir bar, 50 mg of lignin, 10 mL 2 M aqueous sodium hydroxide, and 3.3 mg CuSO₄ • 5 H₂O. The solution was stirred at room temperature until the lignin dissolved while 115 mL of water as a heating medium was added to a 1-L, stainless steel Parr reactor. The PTFE tube containing the reaction contents, as well as up to 7 other prepared reactions, were arranged in the 1-L stainless steel vessel. The Parr vessel was wrapped with a heating mantle, affixed to a stir plate, sealed with a lid bearing a pressure gauge and thermocouple, then protected with a blast shield. The stirring was turned on and the reactor was pressurized to 25 bar with air. The heating mantle and thermocouple were connected to a Parr 4838 Reaction Controller tuned to a 175 °C set point and turned on to heat the reactions. After 45 minutes, when the reaction reached 160 °C, the heating was turned off and the reactor was submerged in a bucket of ice. When the reaction temperature fell to below 45 °C, the pressure was released and the reactor was opened. The contents of the PTFE tube were acidified with conc HCl until the solution became cloudy and extracted with ethyl acetate (3 x 5 mL). The ethyl acetate solutions were combined and concentrated by rotary evaporation until a dark residue remained.

Screening conditions followed the previously described procedure identically except that a dedicated 3 M aqueous solution of sodium hydroxide was prepared for the high alkalinity solution, whereas the 1 and 1.5 M NaOH solutions were generated by diluting 5 mL of the 2 M stock solution with 5 mL deionized water or 7.5 mL of the 2 M stock solution with 2.5 mL deionized water respectively. Copper concentrations were varied by weighing and adding 0, 1.6, or 5 mg of CuSO₄ • 5 H₂O to the reactions, corresponding to 0-, 1-, and 3-mM Cu concentrations respectively (Fig 2b).

Reactions are conducted in duplicates and the yields are averaged.

S3
Figure S1. Reactor set up for lignin oxidative alkaline depolymerization. Left - PTFE vessels are arranged in the 1-L Parr vessel. Right – The fully assembled Parr vessel seated in a heating mantel atop a stirplate.

4. Procedure for HPLC analysis and calibration curve development

The residue to be analyzed was dissolved in a minimal quantity of methanol and transferred to a 10 mL volumetric flask. The contents of the volumetric flask were diluted to the mark and agitated to ensure solution homogeneity. The solution was filtered through a 0.45-micron PTFE syringe filter and 500 μL were transferred to an HPLC vial. A separate 10 mM internal standard solution was made by dissolving 690.8 mg (5 mmol) of 1,4-dimethoxybenzene in methanol and diluting to the mark of a 500 mL volumetric flask. Every sample added to an HPLC vial to be analyzed was further diluted with 500 μL of the 10 mM 1,4-dimethoxybenzene internal standard solution. Samples were eluted using 0.1% formic acid in Milli-Q water and HPLC grade acetonitrile as the A and B mobile phases respectively. Beginning at 5% B, the mobile phase was pumped at 1.5 mL/min with the following gradient profile:
Table S1. HPLC gradient profile for lignin monomer quantitation

| Time (min) | %B mobile phase |
|-----------|----------------|
| 1         | 5              |
| 10        | 7              |
| 20        | 9              |
| 40        | 30             |
| 45        | 35             |
| 50        | 90             |
| 53        | 90             |
| 55        | 5              |
| 60        | 5              |

A calibration curve stock solution was generated by adding the following materials to a 25 mL volumetric flask:

Table S2. Mass of each solute used to generate the most concentrated calibration curve point and serial dilution source.

| Compound               | Mass (mg) | Retention Time (min) |
|------------------------|-----------|----------------------|
| p-hydroxy benzoic acid | 69.8      | 10.3                 |
| vanillic acid          | 85.4      | 14.9                 |
| syringic acid          | 100.2     | 18.6                 |
| vanillin               | 76.3      | 21.6                 |
| syringaldehyde         | 92.1      | 27.3                 |
| acetovanillone         | 83.6      | 28.4                 |
| acetosyringone         | 99.2      | 31.3                 |

The mixed sample was dissolved and the volumetric flask diluted to the mark with methanol, ensuring the final concentration of each solute is ~20 mM. Serial dilution of the stock solution provided 15, 10, 5, 1, and 0.5 mM solutions, each of which was filtered through a 0.45-micron syringe filter. 500 μL of each filtered solution was added to an HPLC vial containing 500 μL of 10 mM 1,4-dimethoxy benzene internal standard solution. Using the previously described gradient profile, the following calibration curves were generated with peak integration performed at 280 nm (±0 bandwidth):
5. General procedure for measuring partition coefficients ($K_p$)

To a 100 mL jar was added solvents in accordance to the ratios dictated by the solvent system to be assessed (Table S3 and S4). The jar was sealed and agitated to ensure equilibration of the solvents. While the biphasic boundary settled, 1 mg of the solute to be assessed was added to a 15 mm x 125 mm test tube. To the test tube were transferred 2 mL of each layer from the biphasic solution in the jar and the test tube was agitated until the solute dissolved and an emulsion was formed. After the emulsion settled, the layers from the test tube were separated and filtered through a 0.45-micron syringe filter into an HPLC vial which was quickly sealed and placed into the chilled HPLC autosampler tray to minimize evaporation. The procedure is visualized in Figure S3. The ratios of absorbances for the solute from each layer provided $K_p$ (Table S5 and S6). Note: formal quantitation is not required since identical solutes under identical separation conditions will have an identical molar absorptivity and flow cell path length. Each mixed solvent system was used for assessing $K_p$ for each solute. The above procedure was repeated for each solvent system to be assessed (Figs 3a and 3b).

Table S3. Volume of each solvent used in generating Arizona Systems for measuring $K_p$.

| Arizona System | Volume (mL) |
|---------------|-------------|
|               | pentane     | ethyl acetate | methanol | water |
| J             | 6           | 15            | 6        | 15    |
| K             | 8           | 16            | 8        | 16    |
| L             | 10          | 15            | 10       | 15    |
| M             | 10          | 12            | 10       | 12    |
| N             | 12          | 12            | 12       | 12    |
**Table S4.** Volume of each solvent used in generating halogenated systems for measuring $K_P$.

| Solvent System | Volume (mL) |
|----------------|-------------|
|                | chloroform  | dichloromethane | acetonitrile | methanol | water |
| ChAW           | 25          | -               | 15           | -        | 10    |
| ChMW           | 25          | -               | -            | 15       | 10    |
| DiAW           | -           | 25              | 15           | -        | 10    |
| DiMW           | -           | 25              | -            | 15       | 10    |

**Figure S3.** Shake-flask workflow used to determine $K_P$ for each solute.

**Table S5.** $K_P$ and log($K_P$) values for each solute in both sets of solvent systems.

| $K_P$            | J    | K    | L    | M    | N    | ChAW  | ChMW  | DiAW  | DiMW  |
|------------------|------|------|------|------|------|-------|-------|-------|-------|
| pHBA             | 3.498| 2.054| 0.914| 0.452| 0.205| 1.075 | 2.831 | 0.889 | 4.400 |
| Vanillic acid    | 2.667| 1.430| 0.723| 0.351| 0.159| 0.545 | 1.089 | 0.395 | 1.992 |
| Syringic acid    | 1.309| 0.781| 0.379| 0.191| 0.102|       |       |       |       |
| Vanillin         | 5.015| 2.835| 1.558| 0.831| 0.519|       |       |       |       |
| Syringaldehyde   | 2.522| 1.577| 0.821| 0.445| 0.264| 0.048 | 0.150 | 0.049 | 0.247 |

| log($K_P$)       | J    | K    | L    | M    | N    | ChAW  | ChMW  | DiAW  | DiMW  |
|------------------|------|------|------|------|------|-------|-------|-------|-------|
| pHBA             | 0.544| 0.313| -0.039| -0.344| -0.688|       |       |       |       |
| Vanillic acid    | 0.426| 0.155| -0.141| -0.455| -0.797|       |       |       |       |
| Syringic acid    | 0.117| -0.107| -0.421| -0.719| -0.993|       |       |       |       |
| Vanillin         | 0.700| 0.453| 0.193| -0.080| -0.285|       |       |       |       |
| Syringaldehyde   | 0.402| 0.198| -0.086| -0.352| -0.578| -1.322| -0.823| -1.310| -0.607|
**Figure S4.** The similarities in the physical properties of vanillin and syringaldehyde compared to their acetophenone analogues lead to log($K_P$) differences too small to resolve the compounds by CPC in either set of solvent systems.

**Table S6.** $K_P$ and log($K_P$) values for acetophenone and aldehyde derivatives in relevant solvent systems.

|                  | K    | L    | M    | DiMW |
|------------------|------|------|------|------|
| vanillin         | 2.835| 1.558| 0.831| 0.336|
| acetovanillone   | 2.673| 1.406| 0.817| 0.279|
| syringaldehyde   | 1.577| 0.821| 0.445| 0.238|
| acetoxyringone   | 1.365| 0.774| 0.416| 0.198|

|                  | K    | L    | M    | DiMW |
|------------------|------|------|------|------|
| vanillin         | 0.453| 0.193| -0.080| -0.474|
| acetovanillone   | 0.427| 0.148| -0.088| -0.554|
| syringaldehyde   | 0.198| -0.086| -0.352| -0.624|
| acetoxyringone   | 0.135| -0.111| -0.381| -0.703|

**6. General procedure for screening Arizona solvents on CPC**

Reagent grade pentane, ethyl acetate, methanol, and DI tap water were attached to lines A, B, C, and D respectively and primed. A method file was prepared based on the previously described standard instrument parameters with variation of solvent identity and flow mode taken into account. The Gilson Glider software is encoded with various Arizona solvent ratios that were selected on the Equilibration and Elution tabs when solvent systems were generally assessed. By selecting the desired letter in the System column followed by the upper- and lower-layer options within the Phase column, the correct solvent percentages are automatically filled into the remaining cells. The order of upper- and lower-layer solvents in the Equilibration and Elution tabs as shown below, alongside setting the CPC unit switching valve to the correct mode, allowed selection of ascending (Asc) and descending (Desc) mode separations:
The method file was initialized and the column was allowed to equilibrate. Injection samples consisted of ~50 mg of each compound in the injection and were diluted to approximately 5 mL. Samples dissolved in methanol/water 1:1 were taken up into 10 mL syringes with 0.45-micron syringe filters affixed and attached to the PLC injection port. The injection loop waste line valve was opened, the syringe was depressed to inject the entire sample, and the waste line valve was closed. After the syringe and filter were removed from the PLC, the on-screen prompt was addressed to begin separation. Because fractions were not collected, all analytes were identified based on $\lambda_{\text{max}}$ absorbances (Fig. 3c).

See section 10 (page S12) for additional experimental detail.

## 7. Varying Arizona solvents and flow direction on CPC

Measured $K_P$ values were validated by conducting CPC separations of the mixture of five monomers with the range of assessed solvent systems (excluding AZ J due to the strong preference of all the solutes for the upper organic phase) (Figure S5). We observed improved resolution with increasingly non-polar solvent systems, although experiment duration and solvent consumption became prohibitive in experiments where syringaldehyde was resolved from pHBA.

![Figure S5. CPC traces for the elution of AZ systems K through N conducted in the ascending mode applied to aldehyde and benzoic acid derivatives obtained from lignin oxidative alkaline depolymerization.](image)
Considering the improved resolution of all solutes with increasingly non-polar solvent systems, the same solutes were subjected to separations in the descending mode (Figure S6) in hopes of reducing duration and solvent consumption. Unfortunately, the strong preference of the solutes to partition into the mobile phase led to insufficient stationary phase interaction and thus co-elution of the solutes early in the separation. Additionally, the eluent of these experiments being predominantly water and methanol meant solute recovery would be energy intensive. These experiments reflected the measured $K_p$ values and offered no benefit as demonstrated by the AZ L Desc separation resembling a mirrored AZ L Asc separation. The co-elution suggested a different solvent system would be required to resolve pHBA and vanillic acid.

**Figure S6.** CPC traces for AZ systems K through M conducted in the descending mode applied to aldehyde and benzoic acid derivatives obtained from lignin oxidative alkaline depolymerization.

### 8. Assessment of the 10:6:4 CH$_2$Cl$_2$/MeOH/H$_2$O solvent system

The solvent system was manually generated and equilibrated by combining 2 L of dichloromethane, 1.2 L of methanol, and 800 mL of water in a clean, 4 L, amber glass solvent bottle and agitating it. The layers were separated from one another by decantation and use of a separatory funnel. The first separation demonstrating the success of the system was performed with these solvent layers by attaching bottles of the upper and lower layers to C and B lines respectively. The valve on the CPC rotor unit was set to ascending and the method file was prepared. In addition to the previously described standard method parameters, the method file was prepared with the following Equilibration and Elution parameters:

| Equilibration (Tab) | Start | End | Flow Rate (mL·min$^{-1}$) | System | Phase | Rotor (RPM) | %A | %B | %C | %D |
|---------------------|-------|-----|---------------------------|--------|-------|-------------|----|----|----|----|
| 1                   | Init. | 00 s| 100.0                     | -      | Lower | 500         | 00 | 100| 00 | 00 |
| 2                   | 00 s  | 12:00| 100.0                     | -      | Lower | 500         | 00 | 100| 00 | 00 |
| 3                   | 12:00 | 24:00| 20.0                      | -      | Upper | 900         | 00 | 00 | 100| 00 |
The method file was initialized and the column was allowed to equilibrate. The injection sample consisted of ~50 mg each of syringaldehyde, vanillic acid, and pHBA and was diluted to approximately 5 mL. The sample, dissolved in a minimal quantity of methanol/water 1:1, was taken up into a 10 mL syringe with a 0.45-micron syringe filter affixed and attached to the PLC injection port. The injection loop waste line valve was opened, the syringe was depressed to inject the entire sample, and the waste line valve was closed. After the syringe and filter were removed from the PLC, the on-screen prompt was addressed to begin separation. Because fractions were not collected, all analytes were identified based on $\lambda_{\text{max}}$ absorbances (Fig. 3d).

9. NMR analysis of the 10:6:4 CH$_2$Cl$_2$/MeOH/H$_2$O solvent system

Once it was determined the solvent system was effective in separating the desired solutes, three NMR tubes were prepared with 500 $\mu$L of acetonitrile-d$_3$. To the first tube was added a single drop of the upper layer, to the second tube was added a single drop of the lower layer, and the third tube was sealed without additives as a standard for analyzing the deuterated solvent water content. Each NMR sample was submitted to quantitative $^1$H NMR analysis with identical parameters on a Bruker AVANCE 400 NMR spectrometer. The following relative integrals were reported for each solvent (methanol integrals were summed):

Table S7. Reported integrals for each signal in the NMR acquisitions for both layers.

|                | CH$_2$Cl$_2$ | CH$_3$OH | H$_2$O | CH$_3$D$_3$,CN |
|----------------|--------------|----------|--------|----------------|
| Upper Layer    | 16.2536      | 6.2292   | 1.00   | 0.1706         |
| Lower Layer    | 1.00         | 19.6794  | 18.1663| 0.2817         |
| Blank          | -            | -        | 1.00   | 2.127          |

The ratio of water to the acetonitrile-d$_3$ residual signal in the blank sample was used to calculate the correction to be subtracted from the upper- and lower-layer water signals. Once the values for water were adjusted, the molar proportion of each solvent was determined in the upper and lower layers which were then used with molecular weights and densities to calculate the volume required of each solvent to generate each layer without prior equilibration and separation of solvents. The exact values were rounded to those seen in Figure S7 as the Glider software was not capable of accommodating decimal values.
Figure S7. NMR traces of equilibrated upper and lower layers from 10:6:4 CH$_2$Cl$_2$/CH$_3$OH/H$_2$O in acetonitrile-d$_3$. Integrations were used to determine the molar ratios of each solvent which were then used to code solvent system ratios of each layer into the Gilson Glider software for future automation.

### 10. Purity and recovery assessment of Arizona L Asc separations

The workflow process for separation and recovery analysis of a model sample oxidative lignin depolymerization mixture or an authentic lignin depolymerization mixture is shown in Figure S8.

**Figure S8.** Recovery and purity assessment workflow for AZ L Asc separation. An identical workflow was used in assessing the 10:6:4 CH$_2$Cl$_2$/CH$_3$OH/H$_2$O Asc separation.
CPC Setup and Separation Protocol:
HPLC grade pentane, HPLC grade ethyl acetate, HPLC grade methanol, and Milli-Q water were attached to lines A, B, C, and D respectively and primed. The valve on the CPC rotor unit was set to the ascending mode and the method file was prepared. In addition to the previously described standard method parameters, the method file was prepared with the following equilibration, elution, and collection parameters:

| Equilibration (Tab) | Start | End   | Flow Rate | System | Phase  | Rotor | %A | %B | %C | %D |
|---------------------|-------|-------|-----------|--------|--------|-------|----|----|----|----|
| 1                   | Init. | 00 s  | 100.0     | L      | Lower  | 500   | 00 | 14 | 33 | 53 |
| 2                   | 00 s  | 12:00 | 100.0     | L      | Lower  | 500   | 00 | 14 | 33 | 53 |
| 3                   | 12:00 | 24:00 | 30.0      | L      | Upper  | 1300  | 46 | 50 | 03 | 01 |

| Elution (Tab) | Start | End   | Flow Rate | System | Phase  | Rotor | %A | %B | %C | %D |
|---------------|-------|-------|-----------|--------|--------|-------|----|----|----|----|
| 1             | Init. | 00 s  | 30.0      | L      | Upper  | 1400  | 46 | 50 | 03 | 01 |
| 2             | 00 s  | 70:00 | 30.0      | L      | Upper  | 1400  | 46 | 50 | 03 | 01 |
| 3             | 70:00 | 82:00 | 100.0     | L      | Lower  | 500   | 00 | 14 | 33 | 53 |

| Collection (Tab) | Time | Local | Volume | Mode | Action |
|------------------|------|-------|--------|------|--------|
| 1                | 00 s | No    | Full   | Waste| None   |
| 2                | 4:00 | No    | Full   | All  | None   |

The method was initiated, flushing the column with fresh lower layer stationary phase in preparation of the column (Equilibration tab, Row 2). At the end of Row 2, pumping halted while the rotor accelerated to the RPM value set in Row 3, during which the waste line was directed into a 1 L graduated cylinder to monitor stationary phase displacement during equilibration. When the rotor reached the intended RPM, mobile phase began pumping (Equilibration tab, Row 3) and column backpressure increased as emulsions formed in each cell and stationary phase was displaced. When mobile phase solvent began eluting from the waste line, the column pressure plateaued at 59 bar. The waste line was directed back into the waste container and the displaced stationary phase was quantified by reading the graduated cylinder marks to the biphasic boundary. The parameters led to 195 mL of stationary phase displaced, corresponding to 80.5% column efficiency or stationary phase retention (retained stationary phase / total column volume). This stationary phase was used to dissolve and dilute the injection sample in a 10 mL volumetric flask.

When equilibration finished, the method paused to allow injection and fraction collection rack preparation. The injection solution from the volumetric flask was taken up into a 10 mL syringe which was then fitted with a 0.45-micron PTFE syringe filter and affixed to the PLC injection port. The injection loop valve was opened, 8.5 mL of the sample was injected, the valve was closed, and elution was initiated in the software prompt (Elution tab, Row 1), reaching a maximum pressure of 66 bar after the rotor reached its target speed. While the separation proceeded (Elution tab, Row 2), a 500 μL sample of the injection solution remaining in the syringe was filtered and transferred into a 5 mL volumetric flask, which was also diluted to the mark. The contents of the 5 mL volumetric flask were filtered through a 0.45-micron PTFE syringe filter and 500 μL of the filtered solution was added to an HPLC vial containing 500 μL of 10 mM 1,4-dimethoxybenzene in methanol. The HPLC sample was submitted to the previously described HPLC analysis.

At the end of the separation extrusion phase (Elution tab, Row 3), a prompt was selected to end the run followed by a prompt to slow and halt the rotor. Fractions corresponding to vanillin, syringic acid, and the
co-eluting compounds were identified based on their $\lambda_{\text{max}}$ values. The contents of the following fraction tubes were pooled and the tubes were rinsed with ethyl acetate to ensure quantitative recovery (Table S8):

**Table S8.** Pooled fractions for quantitative model and lignin sample injections for AZ L Asc separation. Characteristic max absorbances for compound identification included.

| Compound                  | $\lambda_{\text{max}}$ (nm) | Fractions Pooled (synthetic) | Fractions Pooled (authentic) |
|---------------------------|------------------------------|------------------------------|-----------------------------|
| vanillin                  | 275, 305                     | 19-38                        | 27-42                       |
| pHBA Mixture: vanillic acid syringaldehyde | 254                          | 39-79                        | 46-79                       |
|                           | 259, 290                     |                              |                             |
|                           | 307                          |                              |                             |
| syringic acid             | 272                          | 86-109                       | 96-109                      |

The vanillin fractions were concentrated by room temperature rotary evaporation until a volume of approximately 25 mL then stirred with some magnesium sulfate to remove any water. The solution was filtered, the magnesium sulfate was rinsed with additional ethyl acetate, and the pooled solution was concentrated by rotary evaporation to dryness in a tared round bottom flask, then the residue was vacuum dried to constant weight.

The pooled fractions of the pHBA/vanillic acid/syringaldehyde mixture were concentrated by room temperature rotary evaporation in a tared round bottom flask to dryness and the residue was vacuum dried to constant weight.

Because syringic acid eluted during the extrusion phase and contained significant quantities of water, the solution was first concentrated by rotary evaporation at room temperature to no less than 100 mbar. When the solution remained constant in volume and solvent was no longer collecting in the solvent trap, the rotary evaporation vacuum pressure was raised to 400 mbar and the bath temperature was elevated to 40 °C. Once the bath temperature stabilized, the pressure in the rotary evaporator was slowly lowered in 25 mbar intervals with attention focused on the solution to avoid bumping until the sample was dry.

**HPLC Analysis:**
Each residue was weighed, then dissolved in a minimal quantity of methanol. Each solution was transferred to a dedicated 10 mL volumetric flask and diluted to the mark. Each solution was then diluted again to avoid signal saturation by transferring 500 μL into 5 mL volumetric flasks, adding methanol to the mark, and agitating. The contents of each 5 mL volumetric flask were filtered with 0.45-micron PTFE syringe filters and 500 μL of each were added to their own HPLC vial, each containing 500 μL of 10 mM 1,4-dimethoxybenzene in methanol. All vials were subject to the same HPLC analysis described previously. Recoveries were computed as the fraction of material recovered from the original quantity injected while purity was determined as the mass recovered divided by the total mass of the weighed residues. Purity and recovery were not determined for the mixed fractions, but the sample was quantified before injection into the second CPC separation. See the Excel spreadsheet ESI† for a demonstration of calculations.

11. **Purity and recovery assessment of 10:6:4 CH$_2$Cl$_2$/MeOH/H$_2$O Asc separations**
Dichloromethane, HPLC grade methanol, and Milli-Q water were attached to lines B, C, and D respectively and primed. The valve on the CPC rotor unit was set to the ascending mode and the method file was
prepared. In addition to the previously described standard method parameters, the method file was prepared with the following equilibration, elution, and collection parameters:

**Equilibration (Tab)**

| Start | End   | Flow Rate | System | Phase   | Rotor | %A  | %B  | %C  | %D  |
|-------|-------|-----------|--------|---------|-------|-----|-----|-----|-----|
| 1     | Init. | 00 s      | 100.0  | M       | Lower | 500 | 00  | 87  | 11  | 02  |
| 2     | 00 s  | 12:00     | 100.0  | M       | Lower | 500 | 00  | 87  | 11  | 02  |
| 3     | 12:00 | 24:00     | 25.0   | M       | Upper | 1000| 00  | 08  | 51  | 41  |

**Elution (Tab)**

| Start | End   | Flow Rate | System | Phase   | Rotor | %A  | %B  | %C  | %D  |
|-------|-------|-----------|--------|---------|-------|-----|-----|-----|-----|
| 1     | Init. | 00 s      | 25.0   | M       | Upper | 1100| 00  | 08  | 51  | 41  |
| 2     | 00 s  | 35:00     | 25.0   | M       | Upper | 1100| 00  | 08  | 51  | 41  |
| 3     | 35:00 | 48:00     | 100.0  | M       | Lower | 500 | 00  | 87  | 11  | 02  |

**Collection (Tab)**

| Time  | Local | Volume | Mode  | Action |
|-------|-------|--------|-------|--------|
| 1     | No    | Full   | Waste | None   |
| 2     | No    | Full   | All   | None   |

The method was initiated, flushing the column with fresh lower layer stationary phase in preparation of the column (Equilibration tab, Row 2). At the end of Row 2, pumping halted while the rotor accelerated to the RPM value set in Row 3, during which the waste line was directed into a 1 L graduated cylinder to monitor stationary phase displacement during equilibration. When the rotor reached the intended RPM, mobile phase began pumping (Equilibration tab, Row 3) and column backpressure increased as emulsions formed in each cell and stationary phase was displaced. When mobile phase solvent began eluting from the waste line, the column pressure plateaued at 61 bar. The waste line was directed back into the waste container and the displaced stationary phase was quantified by reading the graduated cylinder marks to the biphasic boundary. The parameters led to 215 mL of stationary phase displaced, corresponding to 78.5% column efficiency or stationary phase retention (retained stationary phase / total column volume). This stationary phase was used to dissolve and dilute the mixed fraction sample in a 10 mL volumetric flask.

When equilibration finished, the method paused to allow injection and fraction collection rack preparation. The pHBA/vanillic acid/syringic acid solution from the volumetric flask was taken up in a 10 mL syringe which was then fitted with an 0.45-micron PTFE filter and affixed to the PLC injection port. The injection loop valve was opened, 8.5 mL of the sample was injected, the valve was closed, and the elution was initiated in the software prompt (Elution tab, Row 1) reaching a maximum column pressure of 69 bar after the rotor reached its target speed. While the separation proceeded (Elution tab, Row 2), a 500 μL sample of the injection solution remaining in the syringe was filtered and transferred into a 5 mL volumetric flask, which was also diluted to the mark. The contents of the 5 mL volumetric flask were filtered through a 0.45-micron PTFE syringe filter and 500 μL of the filtered solution was added to an HPLC vial containing 500 μL of 10 mM 1,4-dimethoxybenzene in methanol. The HPLC sample was submitted to the previously described HPLC analysis.

At the end of the extrusion phase (Elution tab, Row 3), a prompt was selected to end the run followed by a prompt to slow and halt the rotor. Fractions corresponding to pHBA, vanillic acid, and syringaldehyde were
identified based on their $\lambda_{\text{max}}$ values. The contents of the following fraction tubes were pooled and the tubes were rinsed with ethyl acetate to ensure quantitative recovery of any solutes:

Table S9. Pooled fractions for quantitative model and lignin sample injections for 10:6:4 CH$_2$Cl/CH$_3$OH/H$_2$O Asc separation. Characteristic max absorbances for compound identification included.

| Compound       | $\lambda_{\text{max}}$ (nm) | Fractions Pooled (synthetic) | Fractions Pooled (authentic) |
|----------------|-------------------------------|-------------------------------|-------------------------------|
| pHBA           | 254                           | 10-15                         | 15-19                         |
| vanillic acid  | 259, 290                      | 17-26                         | 23-29                         |
| syringaldehyde | 307                           | 65-77                         | 77-88                         |

The syringaldehyde fractions were concentrated by room temperature rotary evaporation to dryness in a tared round bottom flask and the residue was then vacuum dried to constant weight.

Because the pHBA and vanillic acid fractions eluted with the mobile phase and contained large quantities of water, they were first concentrated by rotary evaporation at room temperature to no less than 100 mbar. When the solutions remained constant in volume and solvent was no longer collecting in the solvent trap, the rotary evaporation vacuum pressure was raised to 400 mbar and the bath temperature was elevated to 40 °C. Once the bath temperature stabilized, the pressure in the rotary evaporator was slowly lowered in 25 mbar intervals with attention focused on the solution to avoid bumping. Once the samples dried to a residue, the samples were vacuum dried to constant weight.

Each residue was weighed, then dissolved in a minimal quantity of methanol. Each solution was transferred to a dedicated 10 mL volumetric flask and diluted to the mark. Each solution was then diluted again to avoid signal saturation by transferring 500 μL into 5 mL volumetric flasks and adding methanol to the mark and agitated. The contents of each 5 mL volumetric flask were filtered with 0.45-micron PTFE syringe filters and 500 μL of each were added to their own HPLC vials, each containing 500 μL of 10 mM 1,4-dimethoxybenzene in methanol. All vials were subject to the same HPLC analysis described previously. Recoveries were computed as the fraction of material recovered from the original quantity injected while purity was determined as the mass recovered divided by the total mass of the weighed residues.

12. **NMR spectra of solutes recovered from CPC separations**

All NMR data collected in this section for structural confirmation were acquired on a Bruker AVANCE III 500 equipped with a DCH cryoprobe. Each sample was prepared in DMSO-d6 with 1,3,5-trimethoxy benzene as an internal standard.
Figure S9. $^1$H NMR spectrum of vanillin recovered from lignin depolymerization. Small peaks correspond to acetovanillone.

Figure S10. $^1$H NMR spectrum of syringic acid recovered from lignin depolymerization.
Figure S11. $^1$H NMR spectrum of p-hydroxybenzoic acid recovered from lignin depolymerization.

Figure S12. $^1$H NMR spectrum of vanillic acid recovered from lignin depolymerization.
13. Applying one-step 10:6:4 CH₂Cl₂/CH₃OH/H₂O Asc to lignin depolymerization

We attempted to fractionate a lignin depolymerization mixture in one step with the halogenated solvent system by following the same procedure as described in the previous section. All fractions displayed significant impurities, especially for solutes eluting earlier in the separation, and there was insufficient resolution between vanillic and syringic acids. An appropriate fraction to demarcate a boundary between vanillin and syringaldehyde fractions was also difficult to identify due to an impurity eluting between them. Multiple pooled fractions would require a second stage of purification to achieve similar purities to the two-step purification detailed in the manuscript, nullifying any potential benefit. The consumption of significant solvent and necessitated recovery of solutes from high boiling solvents led us away from using the halogenated system as a one-step purification without further optimization.

Figure S13. ¹H NMR spectrum of syringaldehyde recovered from lignin depolymerization. Small peaks correspond to acetylsyringone.
14. Determination of solute enrichment factors

Purities were used to determine the enrichment factor for each solute relative to the first injection. The wt% yields obtained for each solute in the total 1.6 gram lignin injection were assumed to be approximate purities and the final purities for each isolated solute were divided by the yield for each solute.

Table S10. Yields for the injected and purified lignin depolymerization mixture alongside final purities and enrichment factors for each solute.

| Compound                  | Yield (wt%) | Final Purity (%) | Enrichment Factor (Final Purity/Yield) |
|---------------------------|-------------|------------------|----------------------------------------|
| p-hydroxy benzoic acid    | 4.0         | 72               | 18.0                                   |
| vanillic acid             | 1.2         | 81               | 67.5                                   |
| syringic acid             | 2.1         | 50               | 23.8                                   |
| vanillol/acetovanillone   | 5.6         | 65               | 11.6                                   |
| syringaldehyde/acetosyringone | 12.6    | 87               | 6.9                                    |
| Total                     | 25.5        |                  |                                        |

15. Analysis of the remaining collected fractions in lignin sample separation

Whereas all fractions corresponded to detected absorbances in CPC traces, only half of the collected fractions contained known and desired solutes. In agreement, all collected fractions in the first separation showed significant coloration (Figure S10), with the greatest absorbances in the earliest and latest fractions. Such coloration is common in lignin treatments and is suspected to be the result of quinone, quinone methide, and 4,4’-stilbene quinone groups or precursors bound to the lignin polymer. The coloration is likely attributable to such compounds bound to incompletely depolymerized or damaged oligomeric lignin fragments of widely varying identities, rationalizing the presence of these impurities in all fractions. The majority of oligomers elute during the CPC extrusion phase, indicating a strong preference for the water-rich phase, possibly due to significant carboxylic acid content from benzylic oxidation and aromatic ring-opening reactions.

Mostly colorless fractions were collected in the second CPC stage, as fractions with comparatively little absorbance were pooled from the first separation for injection. The purification of a less complex sample in this injection led to the high purities for each monomer obtained from this separation. Still, pHBA and
vanillic acid displayed only 72% and 81% purity while syringaldehyde, which eluted during extrusion, was 87% pure. The impurities can be attributed to either ring-opened networks or chromophore-devoid solvent impurities since the fractions for each solute were colorless, HPLC traces of the solutes showed no small molecule impurities, no insoluble precipitates formed when HPLC samples were made, and the CPC trace showed no additional peaks. High carboxylate content associated with abundant ring-opening fragments could rationalize the lower purity of solutes that eluted closer to the solvent front given the water-richness of the mobile phase.

**Figure S15.** All fractions collected from both stages of lignin sample purification. Fractions pooled for the desired solutes are highlighted with their respective colors.

The remaining fractions were pooled and concentrated as follows:

**Table S11.** Division of the remaining fractions into pooled samples based on the CPC trace for further analysis.

| Peak | Fractions      |
|------|----------------|
| a    | 3-12           |
| b    | 13-19          |
| c    | 20-26          |
| d    | 43-45          |
| e    | 80-95          |
| f    | 110-119        |
| g    | 120-128        |

HPLC analysis of each fraction revealed a multitude of peaks previously overshadowed by the most abundant monomers obtained by lignin oxidative alkaline depolymerization. It is also possible to observe small quantities of the known compounds, highlighted by the color-coded bars set vertically across all traces (Figure S11), which were rejected due to their low concentration and abundance of various impurities. Each
set of pooled fractions contains some quantity of oligomers, although HPLC traces of later fractions show a secondary oligomeric peak eluting earlier and over a broader duration of the analysis. This may be due to the greater solubility of the material in water compared to the sharper oligomer peak that elutes during column washing with 90% acetonitrile, which would corroborate networks of ring-opened aromatics.

**Figure S16.** HPLC analysis of the remaining fractions pooled as shown above. Vertical bars correspond to the known solutes displayed below the CPC trace and are listed in respective order.

16. References

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