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Aromatic monomers analysis for reductive catalytic fractionation of lignin by liquid chromatography with diode array detection

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We use this protocol and it's working

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Disclaimer

This protocol is for research purposes only.

Abstract

An analysis method was developed to quantify the products of lignin derived reductive catalytic fractionation (RCF) by high pressure liquid chromatography paired with diode array detection (HPLC-DAD). This was achieved through chromatographic separation using a mobile phase gradient to separate aromatic analytes on a HPLC reverse phase analytical column.

Guidelines

This protocol utilizes an ultra-high pressure liquid chromatography diode array detection (UHPLC-DAD) system manufactured by Agilent Technologies as referenced in 'Materials'. A similar chromatography and detection system can be utilized; however, some parameter nomenclature may deviate depending on the manufacturer.

Materials

Standards:

- Guaiacol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**W253200
- 4-Methylguaiacol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**W267104
- 4-Ethylguaiacol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**W243604
- 4-Propylguaiacol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**W359807
- 4-Propanolguaiacol **AmBeed** **Catalog #**A161873
- Syringol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**D135550
- 4-Ethylsyringol **AA Blocks** **Catalog #**AA0038V7
- 4-Propanolsyringol **Biosynth** **Catalog #**FD67266
- 4-Propylsyringol **Enamine** **Catalog #**EN300-263267
- 4-Propenylsyringol **Chemspace** **Catalog #**CSC102611715
- Phenol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**PHR1047
- 4-Ethylphenol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**E44205
- Isoeugenol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**I17206
- Methyl Paraben **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**H3647
- 4-Hydroxybenzoic Acid **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**H20059
- p-Coumaric Acid **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**C9008
- trans-Ferulic Acid **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**128708
- Methyl trans-p-Coumarate **Tokyo Chemical Industry** **Catalog #**M2259
- Methyl trans-p-Ferulate **Alfa Aesar** **Catalog #**B22657
- Methylhydrocoumarate **Alfa Aesar** **Catalog #**H64255
- Methylhydroferulate **Tokyo Chemical Industry** **Catalog #**M1803

Reagents:

- Acetonitrile Optima **Fisher Scientific** **Catalog #** A996SK
- Formic acid 98 % pure **Thermo Scientific** **Catalog #** AC147932500
- Methanol Optima **Fisher Scientific** **Catalog #** A454SK

Equipment:

Syringe filters:

Equipment

Syringe Filters, 13 mm PTFE

NAME

syringe filter

TYPE

Cytiva

BRAND

2400

SKU

https://www.cytivalifesciences.com/en/us/shop/lab-filtration/syringe-filters-non-sterile/wwptfe-non-sterile-syringe-filters/acrodisc-syringe-filters-with-universal-membranes-p-36635

LINK

13mm

SPECIFICATIONS

Equipment

syringe filters, 13mm nylon membrane

NAME

syringe filter

TYPE

Cytiva

BRAND

4550

SKU

https://www.cytivalifesciences.com/en/us/shop/lab-filtration/syringe-filters-non-sterile/nylon-non-sterile-syringe-filters/acrodisc-syringe-filters-with-nylon-membrane-p-36371

LINK

13mm

SPECIFICATIONS

Guard column holder:

Equipment

SecurityGuard ULTRA Holder	NAME
guard column holder	TYPE
Phenomenex	BRAND
AJ0-9000	SKU
https://www.phenomenex.com/part?partNo=AJ0-9000	LINK
2.1 to 4.6mm ID	SPECIFICATIONS

Guard column:

Equipment

UHPLC C18 guard cartridge	NAME
guard column	TYPE
Phenomenex	BRAND
AJ0-8782	SKU
https://www.phenomenex.com/part?partNo=AJ0-8782	LINK
2.1 mm ID	SPECIFICATIONS

Analytical column:

Equipment	
Luna HST	NAME
reverse phase analytical column	TYPE
Phenomenex	BRAND
00D-4446-B0	SKU
https://www.phenomenex.com/products/luna-hplc-column/luna-c18-2#order	LINK
2.5 µm / 100 × 2.0 mm / 100 Å	SPECIFICATIONS

UHPLC-DAD system:

Equipment	
1290 Infinity UHPLC	NAME
Ultra-high performance liquid chromatography system	TYPE
Agilent Technologies	BRAND
1290 Infinity UHPLC	SKU
https://www.agilent.com/en/product/liquid-chromatography/hplc-systems/analytical-hplc-systems	LINK

Safety warnings

All chemicals used for this procedure are hazardous. Read the Safety Data Sheet (SDS) for each chemical listed and follow all applicable chemical handling and waste disposal procedures. Manufacturer specific SDS information can be found by following the catalog numbers of compounds in 'Materials' list.

Before start

All solvents and chemicals used are listed in the ‘Materials’ section. These are excluded from in-line references to maintain clarity and keep the steps concise.

Preparation of Mobile Phases

1 Mobile Phases

- To make the aqueous 0.2% formic acid, dilute 2.0 mL of formic acid into 1.0 L of 18.2 MΩ·cm ultrapure water (UPW). Volumetric preparation is optimal.
- Acetonitrile is used as the organic mobile phase.

Note

It is advised to prepare sufficient mobile phase for the entire analysis to reduce the need to add additional mobile phase during an active sequence. Adding mobile phase during an active sequence may cause retention time shifting if the new mobile phase varies in pH or acid concentration. This method uses 4.0 mL of mobile phase per injection, calculate how much mobile phase is needed before beginning analysis.

Preparation of Standards

2 **Standards**

Individual analytes found in 'Materials' were dissolved into methanol at a concentration of 20 g/L and combined to make mixed working stock standards which were further diluted in methanol to create the calibration using the scheme below.

2.1 **Calibration Curve**

Calibration curve preparation: reductive catalytic fractionation products

Calibration level	Concentration (µg/mL) (ppm)	Volume of 1000 µg/mL working solution	Volume of methanol as diluent (µL)	Total volume (µL)
1	1	200 µL of Level 2 (5X)	800	1000
2	5	500 µL of Level 3 (2X)	500	1000
3	10	200 µL of Level 5 (5X)	800	1000
4	25	250 µL of Level 5 (2X)	250	500
5	50	50	950	1000
6	75	75	925	1000
7	100	100	900	1000
8	250	250	750	1000
9	500	500	500	1000

Example calibration curve preparation (click to enlarge)

Note

Reporting limits and linear ranges may vary and should be determined for each instrument and analyte individually. The standard ranges in the table above are suggested starting amounts and may change depending on detector response.

Preparation of Samples

3 **Samples**

- Samples must be filtered through a 0.2 µm or smaller filter prior to injection on the high performance liquid chromatography system (HPLC).
- Samples expected to be over the linear range of the instrument should be further diluted in methanol to be within the calibration range to ensure accurate analysis and avoid carryover.

UHPLC-DAD Analysis

4 **Method Specifications**

Analysis of reductive catalytic fractionation products is performed using an Agilent 1290 series ultra high performance liquid chromatography system with a diode array detector (UHPLC-DAD). Complete method parameters are in the tables below.

Note

This method operates at approximately 450 bar on an Agilent 1290 series ultra high performance chromatography system with a binary pump. This exceeds the maximum pressure of a traditional HPLC such as the Agilent 1100 series. Systems that can operate up to 600 bar such as the Agilent 1260 HPLC can utilize this method.

Binary pump configuration

Flow rate	0.5 mL / min
Maximum pressure	450 bar
Mobile phase A	0.2% formic acid in UPW (v/v)
Mobile phase B	100.0% acetonitrile (v/v)

Gradient configuration

Time (min)	Composition A (%)	Composition B (%)
0.00	95.00	5.00
0.50	95.00	5.00
2.40	72.00	28.00
4.00	40.00	60.00
5.20	40.00	60.00
5.21	5.00	95.00
6.50	5.00	95.00
6.51	95.00	5.00
8.00	95.00	5.00

Defined HPLC parameters (click to enlarge)

Multisampler parameters	
Injection volume	1 µL
Draw speed	100 µL/min
Eject speed	100 µL/min
Wait time after draw	2 sec
Bottom sensing	yes
Column compartment parameters	
Temperature	35 °C
Diode array configuration	
Wavelength:bandwidth (reference)	210.00
	225.00
	240.00
	265.00
	280.00
	310.00
	325.00
Peakwidth	>0.013 min (20Hz)
Spectra	Store All
	190-400 nm
	2.0 nm step

Defined UHPLC-DAD parameters (click to enlarge)

Use the analytical column listed below, as well as associated guard and holder listed in 'Materials'.

Equipment	
Luna HST	NAME
reverse phase analytical column	TYPE
Phenomenex	BRAND
00D-4446-B0	SKU
https://www.phenomenex.com/products/luna-hplc-column/luna-c18-2#order	LINK
2.5 µm / 100 × 2.0 mm / 100 Å	SPECIFICATIONS

Analytical Quality Control

5 Multiple strategies are utilized when performing this analysis to ensure instrument stability and reproducibility.

5.1 **Calibration Curves**

- A minimum of 5 standard levels should be used.
- All compounds must have a correlation coefficient (r^2) of 0.995 or greater using a linear calibration fit and ignoring the origin.

5.2 **Calibration Verification Standards (CVS)**

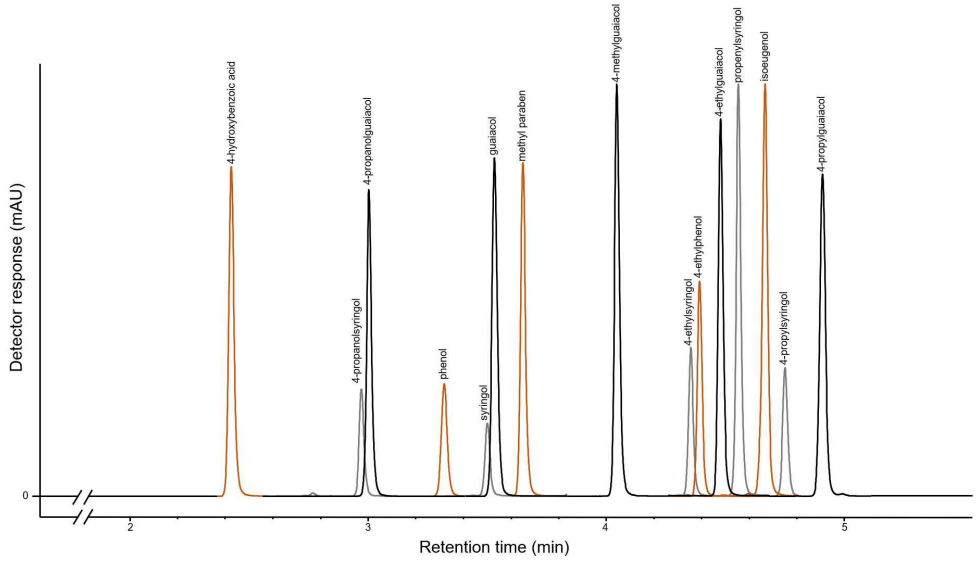
A calibration verification standard (CVS) is a standard from the calibration curve that is re-injected every 20 or fewer samples to ensure instrument drift remains within the determined acceptance criteria. Acceptable CVS recoveries for this analysis are within 10% of the expected amount. Acceptance criteria may differ between instruments and should be determined experimentally.

5.3 **Software of Acquisition and Analysis**

Agilent OpenLab CDS Chemstation Edition Rev C.01.10 [287]

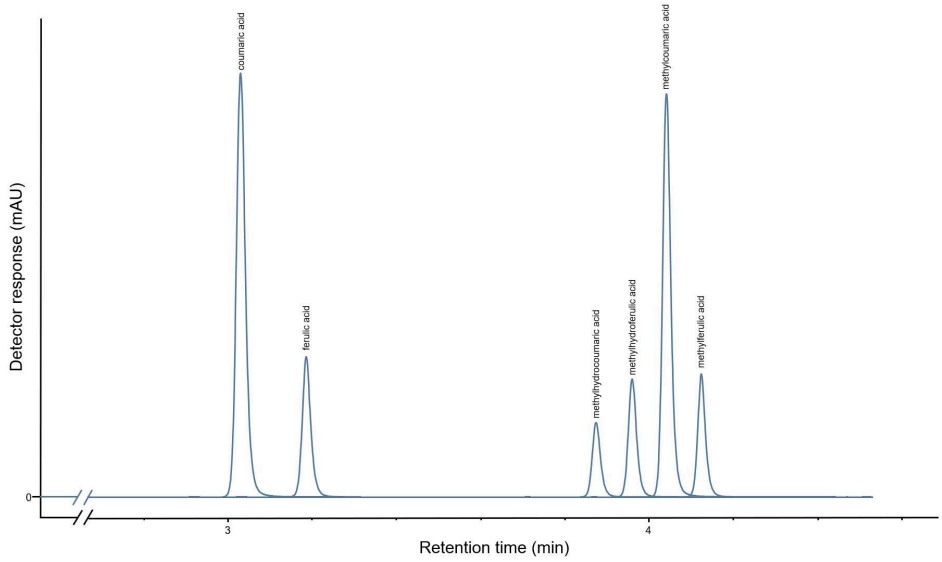
Example Chromatography

6 The chromatogram below illustrates the elution order of analytes quantified for hardwood and softwood substrate experiments.



Example elution order chromatogram. (click to enlarge

Analysis of herbaceous substrate experiments also include the additional analytes in the chromatogram below.



Example elution order chromatogram. (click to enlarge)

Protocol references

Citation

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. Counter-current chromatography for lignin monomer–monomer and monomer–oligomer separations from reductive catalytic fractionation oil.
Green Chem.

https://doi.org/10.1039/D4GC00765D LINK

Citations

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https://doi.org/10.1039/D4GC00765D