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Analysis of monomeric aromatic compounds in alkaline lignin-rich liquors via UHPLC-DAD

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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 aromatic compounds present in lignin-rich, alkaline pre-treated liquors produced from corn stover. This method employs ultra-high pressure liquid chromatography paired with diode array detection (UHPLC-DAD). A reverse phase Luna Omega PS C18 column from Phenomenex and a gradient were used to separate various aromatic compounds.

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

Reagents:

- Protocatechuic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**37580-25G-F
- 4-Hydroxybenzoic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**240141
- Catechol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**PHL82372-100MG
- Ferulic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**128708-5G
- p-Coumaric acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**55823
- Vanillin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**V1104-2G
- Vanillic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**94770-10G
- Vanillyl alcohol **TCI Chemicals Catalog #**V0018
- 4-hydroxybenzaldehyde **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**144088-50G

- Acetonitrile Optima **Fisher Scientific Catalog #** A996SK
- Phosphoric Acid ACS 85 wt. % in Water **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**695017
- Ethanol 99.5% ACS **Thermo Scientific Catalog #** AC615090010

Instrumentation

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

Column

Equipment

Luna Omega PS C18	NAME
UHPLC column	TYPE
Phenomenex	BRAND
00D-4752-AN	SKU
https://www.phenomenex.com/products/luna-omega-hplc-column/luna-omega-ps-c18	LINK
2.1 mm x 100 mm, 1.6 µm	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

Guard holder

Equipment

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

Troubleshooting

Safety warnings

All chemicals used for this procedure are hazardous. Read the Safety Data Sheet (SDS) for all chemicals and follow all applicable chemical handling and waste disposal procedures. Manufacturer specific SDS information can be found by following the CAS 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 standards

1 By weight, create individual 10 g/L stock standards of the following analytes using ethanol (EtOH) as a diluent:

- Protocatechuic acid
- Catechol
- 4-Hydroxybenzoic acid
- p-Coumaric acid
- Ferulic acid
- Vanillyl alcohol
- Vanillic acid
- 4-Hydroxybenzaldehyde
- Vanillin

2 Combine stock standards to make a 1 g/L aromatics mixed standard using EtOH as a diluent. A potential preparation can be made by adding 1 mL of each of the standards and 1 mL EtOH for an overall 10-fold dilution.

Create a calibration curve with a minimum of 5 points using ultrapure water (18.2MΩ·cm) (UPW) as a diluent.

Calibration curve preparation

Calibration level	Concentration (µg/mL) (ppm)	aromatics mixed standard (µL)	UPW (µL)	Total volume (µL)
1	1	100µL of level 3	900	1000
2	5	100µL of level 5	900	1000
3	10	10	990	1000
4	25	25	975	1000
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)

Preparation of mobile phases

3 Mobile phase A consists of 10 mM phosphoric acid in UPW. This can be prepared by adding 0.67 mL of 85% (w/w) phosphoric acid per liter of UPW.

Mobile phase B was acetonitrile.

Sample preparation

4 Confirm that the sample matrix is compatible with instrumentation. The calibration range for this method is between 1.0 µg/mL and 500 µg/mL. Any samples with analyte concentrations expected to be greater than 500 µg/mL should be diluted appropriately to minimize the potential for carryover. Filter all samples through 0.2 µm filtered prior to injection.

UHPLC analysis

5 Analysis is completed on an Agilent 1290 UHPLC system utilizing the following parameters.

Binary pump configuration

Flow rate	0.5 mL / min
Maximum pressure	950 bar
Mobile phase A	10mM phosphoric acid in water (v/v)
Mobile phase B	Acetonitrile (v/v)

Gradient configuration

Time (min)	Composition A (%)	Composition B (%)
0.00	95.00	5.00
0.10	92.50	7.50
1.67	90.00	10.00
2.92	75.00	25.00
4.50	72.00	28.00
4.51	95.00	5.00
8.00	95.00	5.00

Multisampler parameters

Injection volume	0.5 µL
Draw speed	100 µL/min
Eject speed	100 µL/min
Wait time after draw	2 sec
Bottom sensing	on

Column compartment parameters

Temperature	35 °C

UHPLC parameters (click to enlarge)

Diode array configuration	
Wavelength:bandwidth (reference)	210:4 (360:100)
	240:4 (360:100)
	265:4 (360:100)
	280:4 (360:100)
	310:4 (360:100)
	0.00
	0.00
Peakwidth	>0.013 min (20Hz)
Spectra	Store All
	190 - 400 nm
	2.0 nm step

Diode array configuration (click to enlarge)

Analysis was completed utilizing wavelengths 265 nm and 280 nm. Quantitation of these aromatic compounds can be accomplished on multiple wavelengths. Discretion should be used in choosing the quantitative wavelength that produces the optimal response.

Data analysis and quality control

6 Data analysis was completed using Agilent OpenLab CDS ChemStation Edition Rev. C.01.10

The following criteria are employed to ensure reproducibility and stability throughout the analysis:

- All analyte calibration curves must have a correlation coefficient (r^2) of greater than or equal to 0.995. A quadratic or linear fit may be applied with the origin ignored.
- A calibration verification standard (CVS) should be analyzed every 20 or fewer samples. This is a point from the calibration curve re-analyzed throughout the instrument run to check for drift. An acceptable recovery range for this method is +/- 10% of the expected concentration.

Example chromatogram

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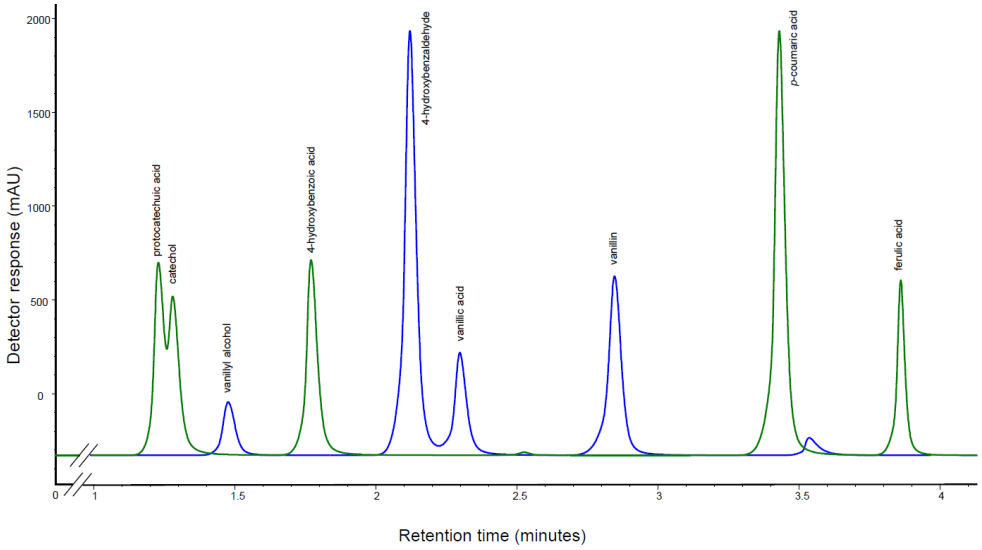

Example chromatogram (click to enlarge)