Oct 16, 2024

Quantitation of aliphatic acids from lignin-derived streams by HPLC-RID

DOI
dx.doi.org/10.17504/protocols.io.rm7vzxrn2gx1/v1

Stefan J. Haugen[1], Alexander F. Benson[1], Sean P. Woodworth[1], David Brandner[1], Kelsey J. Ramirez[1], Gregg T. Beckham[1]

[1]Renewable Resources and Enabling Sciences Center, National Renewable Energy Laboratory, Golden, CO, USA

NREL
Tech. support email: **ftlb_analysis@nrel.gov**

Alexander F. Benson
Renewable Resources and Enabling Sciences Center, National R...

DOI: dx.doi.org/10.17504/protocols.io.rm7vzxrn2gx1/v1

Protocol Citation: Stefan J. Haugen, Alexander F. Benson, Sean P. Woodworth, David Brandner, Kelsey J. Ramirez, Gregg T. Beckham 2024. Quantitation of aliphatic acids from lignin-derived streams by HPLC-RID. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.rm7vzxrn2gx1/v1**

Protocol status: Working
We use this protocol and it's working

Created: January 10, 2024

Last Modified: October 16, 2024

Protocol Integer ID: 93350

Keywords: lignin-derived streams, aliphatic acids, HPLC-RID, oxalic, malic, malonic, succinic, glycolic, lactic, formic, acetic, propionic

Funders Acknowledgement:
This work was authored by the National Renewable Energy Laboratory, operated by Alliance for Sustainable Energy, LLC, for the U.S. Department of Energy (DOE) under Contract No. DE-AC36-08GO28308. This work was supported by Office of Energy Efficiency and Renewable Energy (EERE) and Bioenergy Technologies Office (BETO).
Grant ID: DE-AC36-08GO28308

Disclaimer

This protocol is for research purposes only.

Abstract

An analytical method was developed using high performance liquid chromatography with refractive index detection (HPLC-RID) to quantify the concentrations of aliphatic acids. The analytes quantified were oxalic acid, malic acid, malonic acid, succinic acid, glycolic acid, lactic acid, formic acid, acetic acid, and propionic acid in samples from lignin-derived streams. This method utilized an ion exclusion column to provide chromatographic separation across a 30 minute run-time.

Guidelines

This protocol utilizes an high pressure liquid chromatography equipped with a refractive index detector (HPLC-RID) 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:

Sulfuric Acid 10N Solution **Fisher Scientific Catalog #**SA200-1

Sulfuric Acid, 72% (w/w) **Ricca Chemical Company Catalog #**R8191600-1A

Standards:

Aliphatics Mix 9 components **Absolute Standards Catalog #**67191

Aliphatics Mix 9 components COA.pdf

The *Aliphatics Mix 9 components* standard contains the following compounds.

1. Oxalic acid
2. Malic acid
3. Malonic acid
4. Succinic acid
5. Glycolic acid
6. Lactic acid
7. Formic acid
8. Acetic acid
9. Propionic acid

Consumables:

Syringe filters for aqueous matrices

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

Equipment:

Equipment

Agilent 1260 Infinity II LC System	NAME
HPLC System	TYPE
Agilent	BRAND
Agilent 1260 Infinity II LC System	SKU
https://www.agilent.com/en/product/liquid-chromatography/hplc-systems/analytical-hplc-systems/1260-infinity-ii-lc-system	LINK
G7111B Quat Pump, G7167A 1260 Multisampler, G7116A 1260 MCT, G7117C DAD HS, G7162A 1260 RID	SPECIFICATIONS

Column:

Analytical Column

Equipment

Rezex ROA-Organic Acids H+ (8%)	NAME
LC Column	TYPE
Phenomenex	BRAND
00H-0138-K0	SKU
https://www.phenomenex.com	LINK
300 x 7.8 mm	SPECIFICATIONS

Guard Column

Equipment

Rezex ROA-Organic Acid H+ (8%)	NAME
LC Guard Column	TYPE
phenomenex	BRAND
03B-0138-K0	SKU
https://www.phenomenex.com/	LINK
50 x 7.8 mm	SPECIFICATIONS

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.

Sulfuric acid can cause serious chemical burns. See SDS for additional information: https://beta-static.fishersci.com/content/dam/fishersci/en_US/documents/programs/education/regulatory-documents/sds/chemicals/chemicals-s/S25899.pdf

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 phase and instrument equilibration

1 Mobile phase preparation

- To make 0.02 N sulfuric acid (0.01 M), dilute 2.0 mL of 10 N sulfuric acid into 1.0 L of 18.2MΩ·cm ultrapure water (UPW).

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 is not identical to the original mobile phase. This method uses roughly 15.0 mL of mobile phase per injection. Calculate how much mobile phase is needed before beginning analysis.

2 Instrument equilibration

1. Purge the high performance liquid chromatography (HPLC) instrument with 0.02 N sulfuric acid mobile phase made in step 1. Ensure the instrument is purged through the entire flow path including the detector, before the analytical column is installed.
2. Install the Rezex ROA-Organic Acid LC guard column and the Rezex ROA-Organic Acid analytical column to the system. Begin equilibrating the column at a low flow rate of 0.1 mL/min while the column approaches compartment temperature.
3. Purge the reference cell of the refractive index detector (RID), leave reference cell purge valve open through the duration of the column equilibration.
4. At intervals of at least 10 minutes, increase the flow by 0.2 mL/min until you reach the method flow of 0.5 mL/min.
5. Once the system is at method flow rate and the column compartment and the RID reach analysis temperature, close the reference cell purge valve. This typically takes around 30 minutes after method flow rate is reached. A longer purge of the reference cell is not detrimental to the system.
6. After the reference cell is closed, wait for the RID signal to stabilize before starting analysis.

Preparation of standards

3 Standards

1. Remove aliphatics mix ampule (listed in 'Materials' section) from freezer and allow it to come to room temperature, vortex, and transfer contents of ampule into a 2 mL amber HPLC vial.
2. Using this 5 g/L stock mix, follow the table below to create a calibration curve.

Note

Standards should be kept in a freezer capable of maintaining a temperature of -20°C.

3.1 Calibration curve

Calibration curve preparation

Calibration level	Concentration (µg/mL) (ppm)	Volume of aliphatics mix 9 component (µL)	Volume of UPW as diluent (µL)	Total volume (µL)
1	25	100 µL of level 4	900	1000
2	50	100 µL of level 5	900	1000
3	125	25	975	1000
4	250	50	950	1000
5	500	100	900	1000
6	1000	200	800	1000
7	1250	250	750	1000
8	2500	500	500	1000
9	5000	1000	0	1000

Example calibration curve preparation (click to enlarge)

Acidification procedure

4

Note

For alkaline samples or samples containing high molecular weight lignin the following sample acidification should be performed prior to analysis to avoid column fouling. If samples don't meet these criteria, proceed to step 5 below.

An addition of concentrated acid in excess is needed to lower sample pH and remove components that are harmful to the HPLC guard column and analytical column. This allows for better chromatographic results and subsequently better quantitation of aliphatic acids. Acidification is performed by adding 72% sulfuric acid to samples at a proportion of ~3.5% of the total sample volume.

Define volume of sample to be acidified, noting that filtration will retain approximately 30 µL of liquid. Use the equation below to calculate the volume of acid to be added to each sample

respectively. Example: 1.5 mL of sample requires 52.2 µL of 72% sulfuric acid.

(sample volume[µL]) * (0.035) = (amount of 72% sulfuric acid to be added [µL])

4.1 Add acid at calculated volume to sample in labelled acidification microfuge tube and record sample volume used and vortex thoroughly.

4.2 Affix filtration system (0.2 µm nylon) to luer lock syringe. Add sample to syringe, carefully and **slowly** plunge acidified sample through filter to avoid bursting filter membrane into labelled HPLC vial. Samples are now ready for instrument analysis.

5 Skip step 5 if acidification procedure was performed. Samples must be filtered through a 0.2 µm or smaller filter prior to injection on the HPLC ('Materials' section includes part numbers for filters).

5.1 Samples that are expected to be over the linear range should be diluted to ensure accurate analysis and avoid carryover.

HPLC-RID analysis

6 Prepare an Agilent 1260 Infinity II LC System according to the following parameters for a total run time of 30 minutes.

Binary pump configuration

Flow rate	0.5 mL/min
Maximum pressure	100 bar
Mobile phase A	0.02N sulfuric acid in H_20 (v/v)

Gradient configuration

Time (min)	Composition A (%)	Composition B (%)
0.00	100.00	0.00
30.00	100.00	0.00

Column compartment parameters

Temperature	70 °C

Defined HPLC parameters (click to enlarge)

Reference index configuration

Optical unit temperature	55° C
Signal	>0.2 min (4 s response time) (2.31Hz)

Advanced settings

Analog output	5(%)
Attenuation	500000 nRIU
Signal Polarity	Positive
Automatic zero before analysis	On
Automatic recycling after analysis	Off

Defined HPLC-RID parameters (click to enlarge)

Data analysis and quality control

7 **Data analysis**

- Data analysis completed using Agilent OpenLab CDS, ChemStation Edition version A.01.11.138

7.1 **Calibration curves**

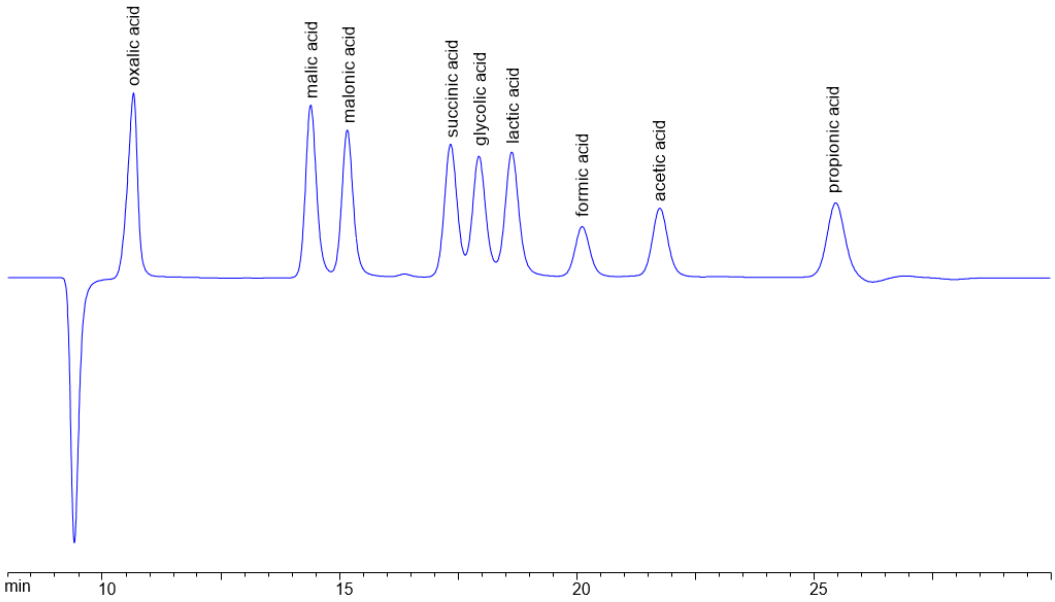
- All compounds must have a correlation coefficient (r^2) of 0.995 or greater using a linear calibration fit and ignore the origin.

7.2 **Calibration verification standard (CVS)**

- A CVS is a standard from the calibration curve that is re-analyzed 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.

Example chromatogram

8



Example chromatogram including elution order of 'Aliphatics Mix 9 components' located in 'Materials'. (click to enlarge)