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Lignin Reductive Catalytic Fractionation (RCF) Monomers Analysis by Gas Chromatography Flame Ionization Detection (GC-FID)

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ABSTRACT

A gas chromatography with flame ionization detection (GC-FID) method was developed to quantify reductive catalytic fractionation (RCF) monomers.

GUIDELINES

NOTICE
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MATERIALS

Woody RCF Standards

Phenol **Contributed by users**
Guaiacol **Contributed by users**
4-Ethylphenol **Contributed by users**
4-Methylguaiacol **Contributed by users**
4-Ethylguaiacol **Contributed by users**
Syringol **Contributed by users**
4-Propylguaiacol **Contributed by users**

Keywords: Reductive Catalytic Fractionation, Gas Chromatography

Isoeugenol **Contributed by users**
Methylparaben **Contributed by users**
4-Ethylsyringol **Contributed by users**
4-Propylsyringol **Contributed by users**
4-Propanolguaiacol **Contributed by users**
Propenylsyringol **Contributed by users**
4-Propanolsyringol **Contributed by users**

Non-Woody RCF Standards

Methyl-3-(4-hydroxyphenyl) propionate **Contributed by users**
Methyl-3-(4-hydroxy-3-methoxyphenyl) propionate **Contributed by users**
Methylcoumarate **Contributed by users**
Methylferulate **Contributed by users**

Internal Standard

Tri-tert-butylbenzene **Contributed by users**

Reagents

Methanol **P212121 Catalog #PA-33900HPLCCS4L**

Equipment

Equipment	
Gas Chromatograph	NAME
8890 GC System	TYPE
Agilent	BRAND
8890 GC System	SKU
https://www.agilent.com/en/product/gas-chromatography/gc-systems/8890-gc-system	LINK

Equipment

HP5-MS	NAME
GC Column	TYPE
Agilent	BRAND
19091S-433UI	SKU
https://www.agilent.com/store/productDetail.jsp?catalogId=19091S-433UI	LINK

Equipment

Inlet Liner	NAME
Agilent	BRAND
5183-4711	SKU
https://www.agilent.com/store/productDetail.jsp?catalogId=5183-4711	LINK
Inlet liner, split, single taper, glass wool, deactivated	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.

BEFORE START INSTRUCTIONS

All solvents, analytes, and chemicals used in this protocol are listed in the 'Materials' section. They are excluded from in-line referencing to keep steps clear and concise.

Internal Standard Preparation

1 This analysis uses 1,3,5-tri-tert-butylbenzene (TTB) as an internal standard. Prepare adequate volume to allow for addition of 1µL of TTB per 100 µL of sample or standard volume.

2 Create a 10,000 µg/mL internal standard working solution by weight of TTB using methanol as the diluent.

Preparation of Standards

3 By weight, create individual 20,000 µg/mL stock solutions of all monomers (listed in Materials section) and use methanol as the diluent.

> Note
>
> If compounds have not previously been analyzed on the column specified in this method, it is necessary to analyze each monomer separately (at a concentration of approximately 100 µg/mL) to determine retention time for each before analyzing a mixture containing all compounds.

4 Combine the stock solutions to create a 1000 µg/mL mixed standard working solution in methanol.

4.1 For example, to prepare a 10 mL mixed standard working solution of woody RCF analytes (distinguished in Materials section), add 500 µL of each of the 20,000 µg/mL stock solutions (14 analytes) and add 3000 µL methanol.

5 Using the mixed standard working solution at 1000 µg/mL, create a calibration curve from 10 µg/mL to 1000 µg/mL with a minimum of five calibration points using methanol as the diluent.

6 Add 1 µL of 10,000 µg/mL TTB internal standard working solution per every 100 µL of calibration standard.
Example: 10 µL into 1000 µL or 5 µL into 500 µL

6.1

Calibration Level	Concentration (µg/mL)	Volume of Mixed Standard Working Solution (µL)	Volume of Methanol (µL)	Volume of Internal Standard (µL)
9	1000	1000	0	10
8	750	750	250	10
7	500	500	500	10
6	250	250	750	10
5	100	100	900	10
4	75	75	925	10
3	50	50	950	10
2	25	25	975	10
1	10	10	990	10

Example Calibration

Sample Preparation

7 **Oil Samples:**

1. Weigh approximately 5-10 mg of oil into a GC vial and record weight.
2. Add a known volume of methanol to each vial containing sample (for example 1 mL).
3. Add 1 µL of 10,000 µg/mL TTB for every 100 µL of methanol added to the sample vial.
   Example: 10 µL into 1000 µL or 5 µL into 500 µL

Liquid Samples:

1. Aliquot a known volume of sample into a GC vial (for example 1 mL)
2. Add 1 µL of 10,000 µg/mL TTB for every 100 µL of methanol added to the sample vial.
   Example: 10 µL into 1000 µL or 5 µL into 500 µL

GC-FID Analysis

8 Analyze samples using an 8890 Agilent Gas Chromatograph (GC) or equivalent equipped with a flame ionization detector (FID) per the method parameters below:

8.1 Method Parameters

Split/Splitless Inlet Parameters:
Inlet Temperature: 280 °C
Injection Volume: 1 µL
Split Ratio: 2:1
Inlet Liner: split, single taper, glass wool, deactivated (see Materials)
Syringe: P/N 5181-8809
Wash Solvent: Methanol

Column:
HP5-MS (see Materials)
Carrier Gas: Helium
Flow Rate: 1 mL/min (constant flow)

Oven Parameters:
Maximum Oven Temperature: 280 °C

	Rate °C/min	Value °C	Hold Time min	Run Time min
Initial		70	2	2
Ramp	10	280	2	25

Detector Parameters:
Detector: FID
Detector Temperature: 300 °C
Air Flow: 400 mL/min
H2 Fuel Flow: 40 mL/min
Makeup Flow: 10 mL/min

Analytical Quality Control

9 Several strategies are utilized when performing this analysis to ensure instrument stability and reproducibility.

9.1 Calibration Curves

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

9.2 Calibration Verification Standards (CVS)

A calibration verification standard (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 15% of the expected amount. Acceptance criteria may differ between instruments and should be determined experimentally.