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Laboratory Analytical Procedure (LAP)

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Renee M. Happs, Anne E. Harman-Ware, Haoxi Ben, and Jack R. Ferrell III

National Renewable Energy Laboratory

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1. Introduction

1.1 Pyrolysis is a thermochemical process that can be used to convert biomass to solid, liquid and gaseous products for use as renewable chemicals and fuels. The liquid fraction, known as "bio-oil" is complex and challenging to characterize, particularly by means of GC/MS, GPC, LC and FT-IR. NMR can analyze whole bio-oil samples and can provide quantitative results to characterize different functional groups or types of carbon present in bio-oil.[1-7]

1.2 Reliable characterization and quantification of carbon functional groups can be used for comparisons between different pyrolysis experimental conditions or different upgrading processes and catalysts, and also allow for comparisons between bio-oils produced at different facilities.

2. Scope

2.1 This procedure has been optimized for the quantification of different carbons in the different functional groups in pyrolysis bio-oils, including carboxyl groups, aromatic C-O, C-C and C-H bonds, six different carbons in levoglucosan, aliphatic C-O and C-C bonds, methoxyl groups, and two different methyl groups.

2.2 The results for different carbon functional groups in bio-oils are reported as functional group %.

3. Terminology

3.1 *Bio-oil:* The crude liquid product of converting solid biomass into a liquid via fast pyrolysis or other thermochemical conversion process.

3.2 *Pyrolysis* – Chemical decomposition of organic materials by heating in the absence of oxygen.

3.3 *Fast Pyrolysis (FP)* – Pyrolysis conducted with rapid heating and short residence time; typically, less than 10 seconds.

3.4 *Catalytic Fast Pyrolysis (CFP)* – Fast pyrolysis conducted in the presence of a catalyst designed to perform partial deoxygenation.

3.5 *Longitudinal Relaxation Time (T1)*: The time constant associated with the process of excited nuclei exchanging energy with the surrounding lattice to return to equilibrium in the direction of the magnetic field, also known as spin-lattice relaxation time. This relaxation rate is also dependent on field strength of the instrument to be used.

3.6 *Pulse delay (D1)*: The pulse delay allowing for full relaxation of excited nuclei, generally following the rule 5*T_1.

4. Significance and Use

4.1 This procedure is used to determine the relative abundance of various types of C functionalities present in different types of pyrolysis bio-oils, including carboxyl groups (C=O), aromatic C-O, C-C and C-H bonds, six different carbons in levoglucosan, aliphatic C-O and C-C bonds, methoxyl groups, and two different aromatic methyl groups. This data can be used to inform how biomass feedstocks or pyrolysis conditions impact the types and relative distribution of products present in bio-oils.

5. Interferences

5.1 Water and solid residue can affect the results; a well-mixed sample is required to provide consistency. Solids can cause shimming issues even in a well-mixed sample and when possible, it should be ensured that particulates are not present. DMSO is the recommended solvent due to its miscibility with water (some bio-oils have up to 50% water). If water content is known to be low (5% or less) in combination with low oxygen content (20% or less) as is the case with some highly upgraded CFP or hydrotreated oils, deuterated chloroform ($CDCl_3$) or deuterated dichloromethane (DCM-d_2) can also be used as a solvent. Consult Happs et al. 2016 for the use of different solvents for different oils depending on water content and polarity differences, and the level of upgrading in oils.[6]

5.2 Changes in the composition of the bio-oil may occur over time as the bio-oil ages. The same sample of bio-oil could potentially have different ^{13}C NMR measurements after storage for long periods of time and heating of the oil should be avoided prior to preparation and analysis. Storage time or age of oil should be noted prior to analysis.

5.4 Overlap between similar functional groups may be present; for example, significant overlap occurs between the aromatic C-C and aromatic C-H regions. However, the overlaps among significantly different functional groups, such as carboxyl groups, aromatic carbons, and aliphatic carbons are very limited. In addition, the methoxyl groups have unique peaks and can be easily identified. Given this, the integration regions for the significantly different functional groups presented below can give quantitative information. If analysis of the sample requires quantifying the amounts of aromatic C-C versus aromatic C-H, it is recommended that a DEPT (Distortionless Enhancement by Polarization Transfer) experiment be performed on the sample in addition to the quantitative carbon experiment in order to determine the exact regions to integrate for corresponding functional groups. A DEPT experiment allows for distinguishing between quaternary, tertiary, secondary, and primary carbons. While it requires additional instrument time, it is imperative for determining the overlap between aromatic C-C and aromatic C-H carbons. Details on collecting DEPT spectra are provided in the appendix (DEPT-135) and in Happs et al. 2016 (DEPT-90). Either DEPT-90 or DEPT-135 may be used.[6]

5.5 It is possible that peaks may be present in the spectra that are from impurities in relaxation reagent $Cr(acac)_3$ or in the solvent. ^{13}C NMR analysis of a blank stock solution of solvent and relaxation reagent may be run without bio-oil sample to account for any background peaks arising from relaxation reagent or other impurities prior to sample analysis and ensure these peaks are not integrated.

5.6 ^{13}C analysis of bio-oil requires a higher level of NMR experience. Analysis should only be performed on an instrument that has well calibrated ^{13}C NMR pulses and other associated parameters, as these can affect the resulting spectrum. An improperly calibrated ^{13}C experiment may result in incorrect peak integrations, baseline distortions, and spectral artifacts that will result in higher error. Consultation with an NMR professional is recommended.

6. Apparatus

6.1 Analytical balance, accurate to 0.1 mg.

6.2 NMR spectrometer using a standard 5mm broadband room temperature probe with ^{13}C observe and ^{1}H decoupling capabilities and applicable software for both NMR data acquisition and processing. Field strengths between 400 and 600 MHz are recommended.

7. Reagents and Materials

7.1 Reagents.

7.1.1 Chromium acetylacetonate ($Cr(acac)_3$), reagent/analytical grade as the relaxation agent. Please read and follow SDS instructions for $Cr(acac)_3$ CAS # 21679-31-2).

7.1.2 The following deuterated solvents can be used depending on pyrolysis oil properties.[6] If solvents do not already contain TMS (tetramethylsilane) as a chemical shift reference, it can be obtained and added to stock solutions (see section 10.1). Please read and follow SDS instructions for all solvents.

a) Deuterated dimethyl sulfoxide ($DMSO\text{-}d_6$), CAS # 2206-27-1
b) Deuterated chloroform ($CDCl_3$), CAS # 865-49-6
c) Deuterated dichloromethane ($DCM\text{-}d_2$), CAS # 1665-00-5

7.2 Materials.

7.2.1 Glass vials of multiple size.
a) Vials for weighing oil – 0.5 dram (e.g., 1.8 mL glass standard opening AS vials with cap).
b) Vials for stock solvent solution – 3 dram (e.g., 11 mL glass vial with Teflon cap).

7.2.2 Glass transfer pipettes.

7.2.3 Pipettes and tips (50 µl, 200 µl and 1000 µl).

7.2.4 5mm OD thin wall precision NMR tubes appropriate for instrument to be used. Ensure appropriate length if using sample automation (e.g., 7 inch height max).

8. Environmental Safety and Health Considerations and Hazards

8.1 Do not inhale or make contact with DMSO-d_6, other solvents, or bio-oils. Working in a ventilated hood and using appropriate personal protective equipment including gloves and safety glasses is strongly recommended.

8.2 Follow all applicable chemical handling procedures and consult SDS for further guidance.

9. Sampling, Test Specimens, and Test Units

9.1 Bio-oil should be allowed to reach room temperature and thoroughly homogenized to obtain a representative sample.

9.2 Exposure to oxygen and heat should be minimized to prevent sample degradation prior to analysis.

9.3 Bio-oil should be soluble in chosen solvent without formation of layers or precipitates during the time required for analyses, check to ensure complete solubility before and after analyses.

10. Procedure

10.1 Solvent Mixture/Stock Solution Preparation.

Prepare a stock solution of deuterated NMR solvent and $Cr(acac)_3$. For raw fast pyrolysis oils and most low-oxygen CFP oils, DMSO-d_6 should suffice. $CDCl_3$ or DCM-d_2 is recommended for hydrotreated or significantly upgraded/low-oxygen-content oils:

10.1.1. Weigh 83.3 ±0.5 mg $Cr(acac)_3$* in 10 mL volumetric flask with cap (*This amount is enough for 30 samples with a final concentration of 5 mg/mL $Cr(acac)_3$ when mixed with bio-oil).

10.1.2. Optional: If the solvent does not contain 0.05% v/v TMS (tetramethylsilane, as a chemical shift reference), add 5 µL TMS to vial, cap, weigh, and proceed to next step immediately.

10.1.3. Dilute with appropriate solvent, ensure $Cr(acac)_3$ dissolves completely. Brief sonication can assist in solubilization of $Cr(acac)_3$.

10.1.4. Transfer stock solution to 3 dram vial with Teflon cap for storage.

10.2 Preparation of bio-oil sample for ^{13}C NMR.

10.2.1. Transfer 200 µl of well mixed bio-oil to tared 0.5 dram vial with cap and weigh to record exact mass of oil (should be approximately 200 ± 25 mg). Some oils are challenging to pipette with conventional micropipettes due to viscosity, or one may not want to damage a pipette with oil vapors. In this case, weighing 200 mg of oil should be prioritized over pipetting. If enough oil is available, users should prepare triplicates of at least a sub-set of samples (e.g. 10% - 3 samples in triplicate if running 30 total samples) for quality control analysis.

10.2.2. Add 300 µl of stock solvent/relaxation reagent (10.1) and mix thoroughly. Ensure no phase separation or precipitate formation.

10.2.3. Transfer contents of vial to appropriate NMR tube for instrument being used.

10.3 NMR Experiment Parameters and Set-up.

Check the NMR spectrometer to ensure that a suitable liquid-state probe is installed; i.e., a tunable, dual broadband probe optimized for ^{13}C detection, is best. It is advisable to ensure that the spectrometer and data acquisition choices (e.g., receiver dead time delay, digital filtering options) are optimized for a flat baseline. Optimization may depend on spectrometer manufacturer and probe style. Consult NMR professional and/or manufacturer support for assistance in minimizing ^{13}C baseline distortions and artifacts.

10.3.1 Follow steps for sample insertion for the instrument to be used. Briefly, insert sample into the spectrometer using a depth gauge appropriate for the instrument/probe to ensure the sample is centered in the coil.

10.3.2 Lock the sample on the deuterated solvent, for example: DMSO-d_6. Tune both ^{13}C and ^{1}H and shim the sample appropriately. Improperly tuned and shimmed samples will result in incorrect analysis.

10.3.3 Critical ^{13}C NMR pulse program and parameters are listed in Table 1 below Spectra shall be collected using these parameters as they have been optimized for quantitation; decoupling parameters are especially critical. Consult with NMR professional to ensure proper parameter set-up.

Table 1. ^{13}C NMR parameters

NMR parameters	
Number of scans	2048 scans
Pulse sequence	1D sequence, ^{13}C observe, ^{1}H decoupling
Pulse angle	90°
Sweep Width; O1 (center)	300 ppm; 120ppm
Acquisition time	1.2 sec
D1 (Relaxation or Recycle Delay)	3 sec
^{1}H Decoupling	Inverse-gated using Waltz-16

10.4 Data Processing and Analysis.

10.4.1 Process the spectra by applying an apodization function to the FID, exponential multiplication is recommended, with line broadening set to 5 Hz, and Fourier transform the data (check with NMR professional if 5 Hz does not seem appropriate, or there are other problems processing data).

10.4.2 Phase the spectrum so that all peaks are positive and symmetrical, using both zero and first order phase corrections.

10.4.3 Reference spectra by assigning the DMSO-d_6 peak at 39.52 ppm or other solvent peak as appropriate. Alternatively, if TMS is present in solution assign it to 0.00 ppm.

10.4.4 Ensure the DMSO-d_6 peak is as symmetrical as possible. Adjust phase again if necessary, ensuring that all peaks are symmetric.

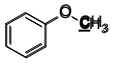
10.4.5 Ensure that baseline issues have been optimized as best as possible on the data acquisition side (see section 10.3.1). Further correct the baseline to remove any remaining artifacts with NMR data processing software. A flat baseline is essential before integration. Use an automatic baseline correction procedure that employs a cubic spline such as Whittaker Smoother, or Bernstein polynomial fit parameter >6, for best results. If you have trouble with baseline correction, consult a NMR professional.

10.4.6 Integrate peak regions based Table 2. The DMSO-d_6 peak shall not be integrated, and the two integration regions on both sides of the DMSO-d_6 peak must be summed as DMSO resonates in the aliphatic region. The sum of the following regions from Table 3 should be normalized to 100%: Carbonyl (230-166.5 ppm), Aromatic (166.5-95.8 ppm, for subcategories please refer to Table 2 depending on

the type of oil being analyzed and/or results from DEPT experiments), Aliphatic C-O (95.8-60.8 ppm), Methoxyl (60.8-55.2 ppm), and Aliphatic C-C (55.2-0 ppm).

Optional: The methyl groups attached to aromatic rings can be further divided if necessary (see Table A3 in the appendix). Also, to quantify levoglucosan, integrate the spectrum for the regions outlined in Table A3, ensuring to individually integrate only the peaks for levoglucosan. The regions for aliphatic C-O and aromatic C-H can still be separated or reported to include levoglucosan, but all integrations still need to sum to 100%, including those regions.

Table 2. ^{13}C-NMR chemical shift assignment ranges for bio-oils.

Type of Carbon (Functional Groups)	Fast Pyrolysis Oil Range (ppm)	Catalytic Fast Pyrolysis Oil Range (ppm)	Functional Group Structure
Carbonyl*	230.0 – 166.5	230.0 – 166.5	
Aromatic C-O	166.5 – 142.0	166.5 – 142.0	
Aromatic C-C	142.0 – 125.0	142.0 – 132.0	
Aromatic C-H	125.0 – 95.8	132.0 – 95.8	
Aliphatic C-O	95.8 – 60.8	95.8 – 60.8	
Methoxyl	60.8 – 55.2	60.8 – 55.2	
Aliphatic C-C**	55.2 – 0.0	55.2 – 0.0	

*Peaks may be overlapped with one of the $Cr(acac)_3$ peaks at ~174.5 ppm, **Peaks may be overlapped with one of the $Cr(acac)_3$ peaks at ~21.74 ppm

10.4.7 Calculate the relative abundance of the peaks in each region relative to the total as shown in Equation 11.1.

10.5 Results.

The following tables can be used as a guide to record data.

10.5.1 Preparation of the stock solution:

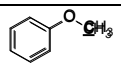
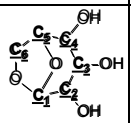
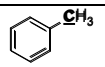
	Volume (ml) / Mass (g)
DMSO-d_6	
$Cr(acac)_3$	

10.5.2 Preparation of NMR sample:

	Volume (ml) / Mass (g)
Stock solution	
Bio-oil	
Total Mass NMR Sample	

10.5.3 Integration report with results shown as functional group %.

Type of carbons (functional groups)	Range (ppm)	Structure	Normalized to 100%
Carbonyl	230.0 – 166.5		
Aromatic C-O	166.5 – 142.0		
Aromatic C-C	142.0 – 125.0 (FP) or 142.0 – 132.0 (CFP)*		
Aromatic C-H	125.0 – 95.8 (FP) or 132.0 – 95.8 (CFP)*		
Aliphatic C-O	95.8 – 60.8		

Type of carbons (functional groups)		Range (ppm)	Structure	Normalized to 100%
Methoxyl		60.8 – 55.2		
Aliphatic C-C		55.2 – 0.0		
Optional	Levoglucosan (peaks are a subgroup of aliphatic C-O and aromatic C-H)	C1 102.3, C2 72.0 C3 73.7, C4 71.7 C5 76.5, C6 64.9		
	Methyl – Aromatic	21.6 – 19.1		
	Methyl – Aromatic’	16.1 – 15.4		

*Requires interpretation and results from DEPT experiment

11. Calculations

11.1 Calculate the functional group % for different carbons in different functional groups:

$$Functional\ Group\ \%\ for\ specific\ C\ type = 100 * \left(\frac{integration\ area\ for\ specific\ type\ of\ C}{sum\ of\ integration\ area\ for\ all\ carbon\ functionalities}\right)$$

*Note**** The aliphatic and aromatic regions must first be integrated with the rest of the functional group regions and those numbers reported on a percentage based on the above calculation. However, the levoglucosan region can then be integrated separately from the functional group regions and included as a subgroup with a percentage less than that of the original aliphatic C-O region. Table A3 shows how the levoglucosan peaks can occupy a large percentage of the aliphatic C-O region. There may be overlap between carbonyl and one of the peaks in $Cr(acac)_3$ and also between aliphatic C-C and another peak in $Cr(acac)_3$ which need to be excluded as carbonyl or aliphatic C-C peaks, respectively. Analysis of the blank stock solution containing $Cr(acac)_3$ can be performed to find if any peaks from the relaxation reagent need to be accounted for and not included in sample spectra integrations and calculations.

12. Report Format

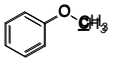
12.1 Report the average amount of carbon in different functional groups per region in bio-oil as functional group %. Standard deviation should also be reported when possible.

13. Precision and Bias

13.1 A well-mixed bio-oil sample is very important for providing a quantitative ^{13}C NMR measurement. Ensure bio-oil is soluble in chosen solvent or try other solvents if in doubt and adjust integrations appropriately. Additionally, concentration gradients may form as sample separates out over time and it is recommended that NMR experiments are run within 24 hours of sample preparation to minimize separation.

13.2 Consistent baseline corrections and integrations of the spectra are essential for accurate and precise analyses, especially when comparing different types of samples. Table 3 shows standard deviations that may be expected from triplicate technical analyses of oils (sampling or homogeneity variation in combination with instrumental and data analyses error) and standard deviation associated with triplicate instrumental analysis of the same exact oil sample by NMR.

Table 3. Typical values and standard deviation associated with sampling (n = 3 samples of the same oil) and instrumental variation (n = 3 analyses of the same oil sample). Two different CFP bio-oils produced under different conditions were tested to demonstrate differences that may be obtained when comparing different oils.

Range (ppm)	Functional Group Structure	FP value and standard deviation (n = 3 samples, normalized to 100%)	CFP value and standard deviation (n = 3 samples, normalized to 100%)	CFP NMR value and instrument standard deviation (n=3 analyses of 1 sample, normalized to 100%)
230.0 – 166.5	 Carbonyl	7.7 (± 0.8)	Oil 1: 4.1 (± 0.4) Oil 2: 5.4 (± 0.4)	Oil 2: 5.4 (± 0.3)
166.5 – 142.0	 Aromatic C-O	9.4 (± 1.0)	Oil 1: 12.5 (± 0.1) Oil 2: 13.0 (± 0.7)	Oil 2: 13.0 (± 0.6)
142.0 – 125.0 or 142.0 – 132.0*	 Aromatic C-C	9.5 (± 0.8)	Oil 1: 9.6 (± 0.2) Oil 2: 8.6 (± 0.4)	Oil 2: 8.6 (± 0.4)
125.0 – 95.8 or 132.0 – 95.8*	 Aromatic C-H	21.5 (± 0.9)	Oil 1: 40.4 (± 0.6) Oil 2: 39.6 (± 1.0)	Oil 2: 39.6 (± 0.6)
95.8 – 60.8	 Aliphatic C-O	28.7 (± 2.7)	Oil 1: 3.0 (±0.5) Oil 2: 3.5 (± 0.3)	Oil 2: 3.5 (± 0.3)
60.8 – 55.2	 methoxyl	3.4 (±0.2)	Oil 1: 0.6 (± 0.1) Oil 2: 1.9 (± 0.2)	Oil 2: 1.9 (± 0.0)
55.2 – 0.0	 Aliphatic C-C	19.8 (± 2.3)	Oil 1: 29.8 (± 0.4) Oil 2: 27.9 (± 1.3)	Oil 2: 27.9 (± 0.4)

*Requires interpretation and results from DEPT experiment

14. Quality Control

14.1 *Reported Significant Figures:* Report results with one decimal place.

14.2 *Replicates:* Prepare triplicate replicates of each sample if possible and analyze each separately on spectrometer using same experimental conditions each time.

14.3 Perform the T_1 test if necessary (refer to the Appendix for more information).

14.4 Perform DEPT-135 (Table A4) or DEPT-90 (Happs et al. 2016) experiment to ensure appropriate integration regions if necessary.[6]

15. Appendices

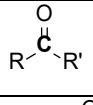
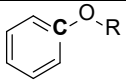
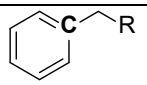
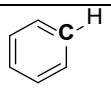
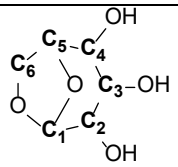
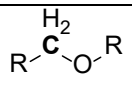
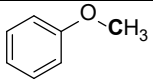
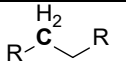
15.1 The T_1 may be different for different samples and may be different at different field strengths; however, as a T_1 measurement requires at least 16-20 hours of NMR time, it is prohibitive to measure T_1 for each sample. Therefore, the recommended pulse delay (D1) for the ^{13}C experiment is set to be >5 times longer than the tested T_1 for the typical bio-oil to ensure quantitative measurement. The use of relaxation reagent such as $Cr(acac)_3$ is recommended in this procedure to reduce T_1 and therefore reduce D1 and overall analysis time, although some interference from peaks in the relaxation reagent has been reported. If spectrometer time is available, a quantitative ^{13}C spectrum may be run overnight (~12-14 hours) without the use of the relaxation agent $Cr(acac)_3$ to analyze the bio-oil samples. In that case, D1 should be at least 10 seconds but may need to be even longer, to ensure that all carbons have fully relaxed, but all other acquisition parameters are the same. The processing of the spectrum is also the same, but elimination of the $Cr(acac)_3$ peaks that may interfere in the spectra is no longer necessary during integration calculations. If necessary and possible, T_1 of the bio-oil sample can be determined using the Inversion-Recovery method and calculate using the standard method in Bruker's TopSpin or other available software.

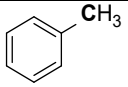
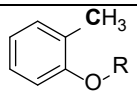
Previous T_1 and integration results for raw FP oils under various parameters are outlined in Tables A1 and A2. The recovery delay list for determination of T_1 was set as: 0.01s. 0.1s, 0.25s, 0.5s, 1s, 2s, 3s, 4s, 8s, 12s, 16s. A concentration of 5 mg/ml $Cr(acac)_3$ gave similar shortened T_1s for all major functional groups and is therefore the recommended value. When using the relaxation reagent, a delay of 3 s was found to be sufficient for pyrolysis bio-oil, as it is still much greater than 5 times T_1, which is normally recommended for quantitative NMR. See Wang et al. 2020 for further discussion on T_1 analysis in bio-oils.[7]

Table A1. The influences of concentrations of relaxation reagent on the T_1 of bio-oils.

	C=O	Aromatic carbons	Aliphatic **C**-O	Aliphatic **C**-C
	230 - 166.5 ppm	166.5 - 95.8 ppm	95.8 - 53.5 ppm	35.3 - 0.0 ppm
5mg/ml $Cr(acac)_3$	459ms	211ms	277ms	269ms
1mg/ml $Cr(acac)_3$	893ms	1244ms	2246ms	785ms

Table A2. Quantitative ^{13}C NMR for 7 aliquots from the same bio-oil sample with 0, 1, and 5 mg/ml relax reagent, different pulse delay (D1), and different number of scans– results presented as functional group %. Variance between different samples may be due to the differences during the sampling process. See Wang et al. 2020 for further discussion.[7]

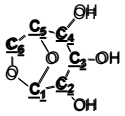
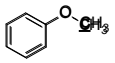
Functional groups	Integration %						
	0 mg/ml d1=60s 4000 scans	1 mg/ml d1=15s 4000 scans	5 mg/ml d1=15s 4000 scans	5 mg/ml d1=3s 4000 scans	5 mg/ml d1=3s 4000 scans	5 mg/ml d1=3s 4000 scans	5 mg/ml d1=3s, with 8000 scans
	16.2%	14.7%	14.0%	14.8%	15.6%	15.3%	13.8%
	7.7%	7.0%	9.3%	9.2%	8.6%	9.5%	10.2%
	3.0%	3.0%	3.4%	2.0%	2.4%	1.8%	3.9%
	12.6%	12.8%	13.1%	13.4%	13.1%	10.9%	13.3%
	30.4%	28.8%	28.8%	30.9%	27.0%	31.0%	28.8%
	40.0%	45.5%	40.7%	44.6%	44.6%	45.9%	43.8%
	3.3%	3.4%	2.8%	3.3%	3.1%	3.6%	4.0%
	17.2%	13.7%	16.7%	12.8%	12.6%	12.9%	11.0%

Functional groups	Integration %						
	0 mg/ml d1=60s 4000 scans	1 mg/ml d1=15s 4000 scans	5 mg/ml d1=15s 4000 scans	5 mg/ml d1=3s 4000 scans	5 mg/ml d1=3s 4000 scans	5 mg/ml d1=3s 4000 scans	5 mg/ml d1=3s, with 8000 scans
	7.3%	6.3%	6.6%	6.9%	5.1%	6.5%	5.1%
	1.0%	0.8%	0.8%	0.6%	0.6%	0.8%	0.5%

Note: D1 has been set to at least 5 times longer than T1 for each experiment.

15.2 Further distinction of functional groups and levoglucosan can be performed by integrating the regions in the ^{13}C spectra as outlined in Table A3.

Table A3. ^{13}C-NMR chemical shift assignment ranges for most fast pyrolysis oils including levoglucosan and methyl groups on aromatic moieties.

Type of carbons (functional groups)	Fast Pyrolysis Oil Range (ppm)	Catalytic Fast Pyrolysis Oil Range (ppm)	Functional Group Structure
Carbonyl*	230.0 – 166.5	230.0 – 166.5	
Aromatic C-O	166.5 – 142.0	166.5 – 142.0	
Aromatic C-C	142.0 – 125.0	142.0 – 132.0	
Aromatic C-H	125.0 – 95.8	132.0 – 95.8	
Levoglucosan	C1 102.3, C2 72.0 C3 73.7, C4 71.7 C5 76.5, C6 64.9	C1 102.3, C2 72.0 C3 73.7, C4 71.7 C5 76.5, C6 64.9	
Aliphatic C-O	95.8 – 60.8	95.8 – 60.8	
Methoxyl	60.8 – 55.2	60.8 – 55.2	
Aliphatic C-C**	55.2 – 0.0	55.2 – 0.0	
Methyl – Aromatic	21.6 – 19.1	21.6 – 19.1	
Methyl – Aromatic’	16.1 – 15.4	16.1 – 15.4	

*Peaks may be overlapped with one of the $Cr(acac)_3$ peaks at ~174.5 ppm, **Peaks may be overlapped with one of the $Cr(acac)_3$ peaks at ~21.74 ppm

15.3 A DEPT-135 experiment may be used to identify appropriate integration regions for aromatic C-C and aromatic C-H. Ensure appropriate phasing and consult NMR facility manager for assistance.

Table A4. DEPT-135 NMR experiment parameters. DEPT-90 may be alternatively used and is detailed in Happs et al. 2016.[6]

NMR parameters	
Number of scans	1024 scans
Pulse sequence	DEPT 135 (DEPT polarization transfer with 135 degree read pulse to give XH, XH3 positive, and XH2 negative with decoupling during acquisition)
Pulse angle	135°
Sweep Width; O1 (center)	300 ppm; 120ppm
Acquisition time	1.2 sec
D1 (Relaxation or Recycle Delay)	3 sec
^1H Decoupling	Recommended is Waltz-16

16. References

[1] Mu, W., Ben, H., Ragauskas, A. & Deng, Y. Lignin Pyrolysis Components and Upgrading Technology Review. *BioEnergy Research* **6**, 1183-1204 (2013).

[2] Ben, H. & Ragauskas, A. J. In Situ NMR Characterization of Pyrolysis Oil during Accelerated Aging. *ChemSusChem* **5**, 1687-1693 (2012).

[3] Ben, H. & Ragauskas, A. J. Heteronuclear Single-Quantum Correlation–Nuclear Magnetic Resonance (HSQC–NMR) Fingerprint Analysis of Pyrolysis Oils. *Energy Fuels* **25**, 5791-5801 (2011).

[4] Ben, H. & Ragauskas, A. J. NMR Characterization of Pyrolysis Oils from Kraft Lignin. *Energy & Fuels* **25**, 2322-2332 (2011).

[5] Braun, S., Kalinowski, H.-O. & Berger, S. in *150 and More Basic NMR Experiments: A Practical Course* Ch. 6, 180-185 (Wiley-VCH, 1998).

[6] Happs, R. M., Iisa, K. & Ferrell III, J. R. Quantitative ^{13}C NMR characterization of fast pyrolysis oils. *RSC Advances* **6**, 102665-102670 (2016).

[7] Wang, R., Luo, Y., Jia, H., Ferrell, J. R., and Ben, H. Development of quantitative ^{13}C NMR characterization and simulation of C, H, and O content for pyrolysis oils based on ^{13}C NMR analysis. *RCS Advances* **10**, 25918-25928 (2020).