Characterization of Lignin by Gas Chromatography and Mass Spectrometry Using a Simplified CuO Oxidation Method

ARTICLE *in* ANALYTICAL CHEMISTRY · NOVEMBER 2011

Impact Factor: 5.64 · DOI: 10.1021/ac202004r · Source: PubMed

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Characterization of Lignin by Gas Chromatography and Mass Spectrometry Using a Simplified CuO Oxidation Method

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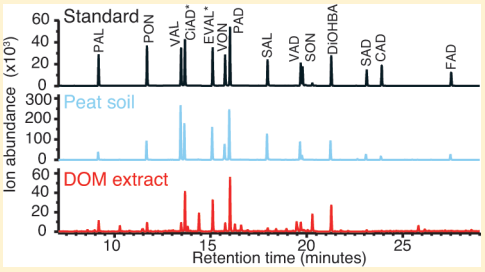
Supporting Information

ABSTRACT: An efficient and high-throughput method to characterize lignin in environmental samples using alkaline CuO oxidation and capillary gas chromatography with mass detection is presented. Monomeric lignin phenols released during oxidation were selectively extracted using a polymer-based solid phase sorbent rather than liquid phase extraction. Sample size and matrix were found to influence lignin phenol yields. Increasing the sample size to an organic carbon content ≥1.5 mg of C minimizes phenol oxidation losses, and the addition of glucose as a sacrificial carbon source helped minimize oxidation losses in samples with <5 mg of organic carbon.



Lignin is a major structural polymer found only in vascular plants. It is embedded in the secondary cell wall and tightly cross-linked to cellulose fibrils and hemicellulose. The rigid secondary cell wall provides plants with conductive tissues for water and nutrient transport and mechanical strength for upright growth. The primary subunits in lignin are coniferyl, sinapyl, and p-coumaryl alcohols.[1] The distribution of these lignin phenols varies with plant group (gymnosperm and angiosperm) and tissue type (woody and herbaceous).[2,3] The chemical stability of the lignin macromolecule and its resistance to microbial degradation results from a variety of hydrolysis-resistant carbon−carbon and ether linkages.[1] Lignin and its degradation products occur in soils, sediments, and natural waters, where it plays an important role in the global carbon cycle.[3−5]

The "classic" CuO oxidation method for lignin characterization in environmental samples was published by Hedges and Ertel[6] almost 30 years ago and has been adopted by numerous research groups.[6] Several drawbacks have limited the wider application of this method. Only four samples can be analyzed during a single oxidation, and the labor-intensive liquid−liquid extraction cleanup procedure with ethyl ether can lead to destruction of oxidation products due to ether peroxides. Since then, several modifications have been described to increase efficiency and sample throughput. Kögel and Bochter[7] presented a silica-based C_{18} solid phase extraction (SPE) procedure to clean up samples after CuO oxidation. A recent method employs a microwave digestion system that allows the simultaneous analysis of six samples.[8] Instead of ethyl ether, ethyl acetate is used for the liquid−liquid extraction procedure. Dalzell et al.[9] and Louchouarn et al.[10,11] reported the use of mini-reaction vessels (3 mL, Prime Focus, Inc.) for the analysis of small sample sizes. For quantification of oxidation products, the original method by Hedges et al.[6] was expanded from gas chromatography (GC) with flame ionization detection to GC/MS and liquid chromatography with UV detection.[7,11−13]

Although the reported changes have led to significant improvements for lignin analysis in environmental samples, many limitations still exist. The microwave oxidation method was developed for sediments, and the large vessels and reaction volumes render it inappropriate for small dissolved organic matter (DOM) samples. In addition, the inefficient liquid−liquid extraction can lead to complex sample mixtures that complicate chromatographic analyses, especially in samples with very low lignin content.[11] Silica-based C_{18} SPE sorbents often suffer from drying out effects, especially on a vacuum manifold, leading to poor performance and recoveries.

In this study, we report a rapid and efficient analytical method for characterization of lignin by CuO oxidation in DOM, soil, and sediment samples. The method includes a 12-sample carousel, a new solid phase extraction cleanup procedure using a polymeric sorbent, and GC/MS quantification of monomeric lignin phenols. Oxidation conditions for degradation of the lignin polymer are also investigated.

EXPERIMENTAL SECTION

Materials. Calibration compounds, cupric oxide, glucose, $Fe(NH_4)_2(SO_4)\cdot 6H_2O$, methyl acetate and *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) mixed with 1% trimethylchlorosilane (TMCS) were obtained from Sigma Chemical Co. (St. Louis, MO). Dry pyridine, methanol, and dichloromethane were purchased from EMD Chemicals (Darmstadt, Germany) and stored over blue silica gel. Anhydrous sodium sulfate and HPLC grade methanol were from J.T. Baker (Phillipsburg, NJ). Oasis HLB extraction cartridges (60 mg, 3 mL) were obtained from Waters (Milford, MA).

Received: August 1, 2011
Accepted: November 17, 2011
Published: November 17, 2011

The extraction manifold (12-port) equipped with inert disposable liners and 1 mL tubes used for drying were from Supelco (St. Louis, MO). Eppendorf repeater pipets were used for pipetting bases and organic solvents. Organic impurities were removed from cupric oxide and sodium sulfate by heating at 450 °C for 3 h. Calibration and internal standards were prepared in dry pyridine. Standards were stored in a freezer (−20 °C) over blue silica gel. All glassware was acid-rinsed and heated to 450 °C for 3 h before use.

Cupric Oxide Oxidation. Cylindrical monel-steel reaction vessels (Figure S-1 in the Supporting Information) were made by Prime Focus Inc. (Seattle, WA) and Service Machine Company (Columbia, SC). Teflon O-rings (5/16 i.d. × 1/16 axial CS) for reaction vessels were bought from Marcorubber (Seabrook, NH). The total volume of a reaction vessel with a loaded steel ball bearing for mixing was 3.2 mL. The original design was developed by John Hedges and Jack Gudeman and manufactured by Lee Bond (Prime Focus Inc.). A steel carousel with cradles for 12 reaction vessels in a ferris-wheel type configuration was mounted in the door of an HP 5890 GC (Figure S-1 in the Supporting Information). The carousel holding the reaction vessels was rotated during the oxidation at 155 °C with an electric motor mounted on the outside of the GC oven door. Steel balls in the reaction vessel ensured samples were mixed continuously during oxidation.

Each reaction vessel was loaded with a steel ball bearing, 330 mg of cupric oxide, 106 mg of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, and a dry sample. Reagent weights were always within ±1 mg. Glucose (10 mg) was added to the mixture if the amount of organic carbon in samples was <5 mg. Vessels were kept in an argon-filled plastic container to exclude molecular oxygen, and reaction vessels were gently sparged with argon before adding 2.5 mL of NaOH (2 mol L^{-1}or 8 v/v%). The sodium hydroxide solution was prepared with argon-sparged deionized water (15 min). The loading procedure was slightly modified for samples of dissolved organic matter (DOM) that were isolated from aqueous samples by solid phase extraction.[8] Methanolic DOM extracts (10−15 mL) were evaporated to dryness in a Savant Speedvac SVC 200 vacuum centrifuge (40 °C) before oxidation. Dried extracts were redissolved by sonication in 1.5 mL of NaOH (2 mol L^{-1}or 8 v/v %). After transfer to preloaded reaction vessels, glass tubes were rinsed twice with 0.5 mL of 2 mol L^{-1}NaOH, and the rinses were added to the reaction vessel. After closing, the reaction vessels were vigorously shaken to ensure steel ball bearings were moving freely inside the vessels.

The oxidation temperature program was the following: start temperature 28 °C, heating at 4.1 °C/min to 155 °C, hold for 150 min. After completion of the oxidation, reaction vessels were removed from the GC oven with asbestos gloves and rapidly cooled under running tap water. Reaction vessels were cleaned with labware detergent (Contrex, Decon Laboratories) and soaked in methanol/50 wt % sodium hydroxide 50/50 v/v % (2 h) between analyses.[14] Metal threads on the reaction vessels were wrapped with Teflon tape before every use to prevent thread seizing. The Teflon O-rings used to seal the reaction vessels were replaced after every oxidation.

Solid Phase Extraction (SPE) Cleanup. The reaction vessels were place upright in a test tube rack and shaken individually before opening to minimize residual liquid in the caps. A mixture of *trans*-cinnamic acid (CiAD) and ethyl-vanillin (EVAL) was spiked into the reaction solution immediately after opening the bombs. Following the internal standard addition, vessels were closed and vigorously mixed. The content of each vessel was transferred to glass tubes. The reaction vessels were rinsed twice with 0.5 mL of argon-sparged 1 M NaOH. The combined solutions were centrifuged to remove unreacted cupric oxide, precipitated cuprous oxide, and other particles. After transferring the supernatant to glass tubes, the sample solution was acidified to a pH of 2 with 6 M sulfuric acid. For sediment samples, any precipitate that formed during acidification was removed by centrifugation.

Oasis HLB cartridges (60 mg, 3 mL) were conditioned twice with 2 mL of methanol and deionized water. Samples were applied to the columns and passed through the resin under gravity flow. Salts and interfering components were removed with three rinses of 0.5 mL of water/methanol 70/30 v/v/%. Cartridges were dried under vacuum for 10 min. Before elution, Teflon needle liners were carefully rinsed with acetone and dichloromethane to remove all traces of water. Lignin phenols were eluted sequentially with three rinses of 0.5 mL of dichloromethane/methylacetate/pyridine 70/25/5 v/v/v% followed by two rinses of 0.5 mL of dry methanol. The solvent mixture was prepared fresh before use. During the first elution step with dichloromethane/methyl acetate/pyridine, HLB cartridges were connected to a cartridge (1 mL) packed with 1 g of anhydrous sodium sulfate. The eluent was pulled through the drying cartridge at a flow rate of 0.5 mL/min, and HLB cartridges and drying cartridges were evacuated for 10 s to remove any residual eluent. Drying cartridges were removed before elution with dry methanol. Elution with methanol was carried out under gravity flow, and residual methanol in HLB cartridges was removed with a short burst of vacuum. After the final elution step, the combined fractions were thoroughly mixed. The solvent mixture was evaporated with a gentle stream of argon directly in the eluent manifold. The drying adapter was made of Teflon tubing (4.5 mm i.d.) fit with luer locks (barb to male) that connected to disposable liners on the eluent manifold. The argon gas flow rate was set to 1.75 L/min with a flow gauge. Samples were redissolved in 50−200 μL of dry pyridine and stored frozen over silica gel. HLB cartridges were discarded after single use. Drying tubes were unpacked and cleaned for repeated use. Frits in drying tubes were discarded.

GC/MS Analysis. GC analysis was carried out using an Agilent 7890 system connected to an Agilent 5975C triple axis mass detector with electron impact ionization. Samples were derivatized with BSTFA/TMCS before analysis. Typically, 15 μL of sample was reacted with 15 μL of BSTFA/TMCS reagent at 75 °C for 15 min. The injection volume was 1−2 μL depending on the concentrations of target compounds. The split was set to 15:1 for dissolved organic matter extracts and 20:1 for soil samples. The injector temperature was set to 300 °C. The septum purge flow was 3 mL/min. Straight liners packed with nondeactivated glass wool were used.

Separation was achieved on a (5%-phenyl)-methylpolysiloxane capillary column (Agilent DB-5, 250 μm × 30 m) with the following temperature program at 1.5 mL/min using helium as the carrier gas: start temperature 100 °C, hold for 1 min, ramp at 4 °C/min to 270 °C, hold for 16 min. The mass detector settings were source temperature 230 °C, quad temperature 150 °C, and auxiliary temperature 280 °C. The detector was autotuned using routines provided with the Agilent Chemstation software. The analysis was carried out in full scan mode (range m/z 50−650) and SIM mode (1 characteristic ion for quantification with 2 qualifier ions, Table 1). Structures were assigned by comparison

Table 1. Symbols, Retention Times, Characteristic Mass Fragments, and Limits of Detection for CuO Oxidation Products

compound	symbol	RRT_{DB5}[a]	major ions (m/z)[b]	MDL (fmol/μL)[c]
cinnamic acid	CiAD	1	131,*161*,205,*220*	
ethyl-vanillin	EVAL	1.106	167,179,*195*,*238*	
p-hydroxybenzaldehyde	PAL	0.672	151,*179*,*194*	71
p-hydroxyacetophenone	PON	0.856	193,*194*,*208*	132
p-hydroxybenzoic acid	PAD	1.172	193,*223*,267,*282*	57
vanillin	VAL	0.986	193,194,*209*,*224*	58
acetovanillone	VON	1.153	193,*208*,223,*238*	49
vanillic acid	VAD	1.441	223,267,*282*,297,*312*	75
syringaldehyde	SAL	1.315	224,*239*,*254*	71
acetosyringone	SON	1.448	223,238,*253*,*268*	104
syringic acid	SAD	1.692	253,297,*312*,327,*342*	103
3,5-dihydroxy-benzoic acid	DiOHBA	1.558	28,*311*,*355*,370	113
p-coumaric acid	CAD	1.749	*219*,249,293,*308*	50
ferulic acid	FAD	2.014	*249*,293,308,*323*,338	184

[a] Retention times are relative to CiAD on an Agilent DB-5 column. [b] Underlined ions are used for SIM quantification. Italicized ions were used to assess peak purity. [c] Determined by replicate analysis (n = 7) of a standard spike (~1 nmol) into 2 mol L^{-1} NaOH, 330 mg of CuO, 10 mg of glucose, and 106 mg of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ and analyzed as described in the method. MDL was calculated by multiplying the sample standard deviation with the Student's t-value.

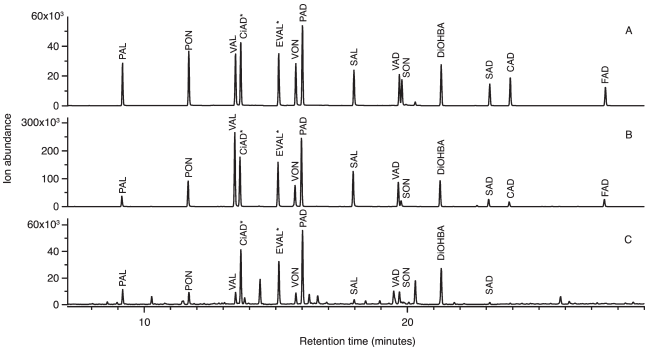


Figure 1. Selected chromatograms of (A) a 40 pmol μL^{-1} standard mixture, (B) peat soil (wt % OC = 42.8), and (C) DOM extract from the Arctic Ocean. DOM extracts were collected on reversed phase sorbents according to Louchouarn et al.[12] Chromatographic conditions and MS detector settings (SIM mode) are described in the text. Abbreviations are explained in Table 1.

with authentic standards and published spectra. Measured compounds were quantified based on one characteristic mass ion and blank corrected.

RESULTS AND DISCUSSION

Chromatography and SIM Quantification. All hydroxyl, vanillyl, syringyl, and cinnamyl phenols (Table 1) were resolved on a 30 m DB-5 capillary column (0.25 μm i.d.) with a linear temperature program. The compound 3,5-dihydroxy-benzoic acid (DiOHBA) was included in the analysis because it is a major CuO oxidation product in environmental samples and was proposed to be an indicator of soil organic matter in marine and freshwater environments.[15,16] Chromatograms of a soil sample and DOM extract compared to a standard mixture are shown in Figure 1. The SPE procedure resulted in clean chromatograms with minimal coelution occurring. This was confirmed by matching characteristic ion ratios determined with standard mixtures and a few nontarget peaks in the full mass scan mode (data not shown). Injection liner packings showed some build-up of nonvolatile compounds and were frequently exchanged (~24 samples) to avoid adsorption effects in the inlet. Peak tailing occurred after ~300 sample injections. Trimming the column (10 cm) and exchange of the gold seal in the injector block typically restored optimal performance. We observed some variability in the ability of DB-5 columns to resolve VAD and SON. Only one out of three columns purchased from the same manufacturer was able to resolve VAD and SON using exactly the same settings, suggesting some variability in column-to-column reproducibility.

The SIM ions were selected based on overall abundance and characteristic fragmentation patterns (Table 1). When VAD and SON coeluted, both compounds could still be quantified due to unique ion fragmentation patterns. Method detection limits (MDL) for individual lignin phenols are shown in Table 1. They were determined by spiking low levels (~1 nmol) of standard mixtures into preloaded reaction vessels and analyzed following the complete analytical procedure. Minimum sample volume after redissolution was 50 μL. The maximum injection volume was 2 μL with a 1:15 split. The mass detector was tuned at the highest possible sensitivity using the Hisense mode in the Agilent software. Effective sample cleanup allowed minimum detection limits of 49−184 fmol μL^{-1} for lignin phenols.

Method blanks were continuously monitored and ranged from 1 to 70 pmol for individual lignin phenols (Table S1 in the Supporting Information). DiOHBA showed a consistently high blank value of 794 ± 111 pmol (n = 6) that was primarily derived from the NaOH solution.[8]

SPE Cleanup and Recoveries. The Oasis HLB resin, a copolymer of divinyl-benzene and *N*-vinyl-pyrrolidone, showed strong retention of lignin phenols without drying effects that are often observed with reversed phase sorbents (Table 2). Continuous drying of HLB sorbent under vacuum for 15 min or any accidental drying during the elution steps had no effect on recovery of lignin phenols. In addition, variations in flow rate during the extraction had no negative impact on retention efficiencies. No breakthrough of lignin phenols occurred even when samples

dx.doi.org/10.1021/ac202004r |*Anal. Chem.* 2012, 84, 459–464

with as much as 1 mmol of organic carbon and 1 μmol of total lignin phenols were loaded onto columns.

Recoveries for all lignin phenols were investigated by spiking dilute standards (~1 nmol for each compound) into preloaded bombs. Multiple transfer steps during cleanup were the greatest source of losses and could be minimized with careful rinsing. Recovery efficiencies were 87−90% for all lignin phenols by employing sequential elution with dichloromethane/methylacetate/pyridine (70/25/5 v/v/v %) and methanol (Table 2).

Table 2. Recoveries of Lignin Phenols

	(%)[a]	relative to CiAD (%)[b]	relative to EVAL (%)[b]
compound	(n = 7)	(n = 6)	(n = 6)
CiAD	89.0 ± 5.9	100.0	101.4 ± 0.8
EVAL	87.8 ± 5.3	98.6 ± 0.8	100.0
PAL	87.9 ± 6.3	98.7 ± 2.2	100.1 ± 2.1
PON	90.2 ± 7.3	101.3 ± 4.2	102.7 ± 4.1
PAD	87.6 ± 5.7	98.4 ± 1.8	99.8 ± 1.5
VAL	87.9 ± 6.2	98.8 ± 1.8	100.2 ± 1.8
VON	88.9 ± 5.9	99.8 ± 1.6	101.2 ± 1.5
VAD	89.4 ± 5.4	100.5 ± 2.4	101.9 ± 2.1
SAL	86.6 ± 6.5	97.2 ± 2.3	98.6 ± 2.3
SON	87.8 ± 7.1	98.6 ± 3.3	100.0 ± 3.4
SAD	88.4 ± 7.3	99.2 ± 3.3	100.6 ± 3.4
DiOHBA	89.2 ± 6.4	100.2 ± 3.6	101.6 ± 3.5
CAD	87.3 ± 5.9	98.0 ± 1.6	99.4 ± 1.6
FAD	88.0 ± 10.9	98.6 ± 5.9	100.0 ± 6.1

[a] Absolute recoveries were calculated from replicates (~1 nmol) run on different days. [b] Recoveries relative to cinnamic acid (CiAD) and ethylvanillin (EVAL) were determined from replicates (~1 nmol) run on the same day.

Corrected recoveries relative to the internal standards CiAD or EVAL were 98−101%, indicating CiAD and EVAL were excellent internal recovery standards, and the SPE method did not introduce any systematic over- or under-estimation of lignin phenol concentrations.

Using a single elution with dichloromethane/methylacetate/pyridine, recoveries were not significantly different for aldehydes and ketones (t test, $p < 0.01$), but the carboxylic acids PAD, VAD, and SAD showed 5−10% lower (Table S-2 in the Supporting Information) recoveries. DiOHBA could not be quantitatively recovered with a single elution due to very strong retention of compounds with more than one hydroxyl group. A single elution with methanol was not feasible as the final extract contained residual water, which was difficult to remove without selective losses of lignin phenols (data not shown).

Absolute recoveries of the internal standards EVAL and CiAD in environmental samples (DOM extracts, peat soils, suspended sediments) monitored over 2 months were 83.0 ± 8.1% (n = 168), and they were not statistically different (t test, $p < 0.001$). Alternating internal standards for quantification typically produced values within the precision of the method (1−12%).

In some samples, EVAL SIM ion ratios deviated from ratios measured in calibration standards suggesting possible coelution. CiAD was also preferred over EVAL as an internal standard because EVAL exhibited the highest volatility of all analyzed phenols and excessive drying of samples after SPE lead to evaporative losses of EVAL. Losses of EVAL were dependent on the sample matrix. Overdrying of recovery standards and blanks resulted in the highest losses of EVAL (10−20%), whereas samples with high carbon loading (>2 mg of OC) showed negligible evaporation of EVAL. The evaporation of phenols was minimized by addition of a small amount of pyridine (5%) to the elution solvent mixture.

Acidification of oxidized samples lead to precipitation of particles. Tests with DOM extracts and peat soil samples showed

Table 3. Concentrations of CuO Oxidation Products in Peat Soil and Dissolved Organic Matter[a]

	peat soil (nmol mg^{-1})	Colville River (nmol L^{-1})	Arctic Ocean surface (pmol L^{-1})	Arctic Ocean deep (pmol L^{-1})	
compound	(n = 5)	(n = 3)	(n = 2)	(n = 2)	mean % deviation
PAL	11.753 ± 0.962	8.387 ± 0.361	223 ± 1	103 ± 5	4.5
PON	14.906 ± 1.038	9.705 ± 0.195	215 ± 0	144 ± 8	3.6
PAD	15.531 ± 1.733	16.252 ± 0.667	501 ± 1	317 ± 8	4.5
VAL	17.414 ± 1.004	4.627 ± 0.058	305 ± 30	56 ± 2	5.0
VON	8.239 ± 0.312	3.101 ± 0.048	152 ± 18	37 ± 2	5.4
VAD	15.021 ± 0.391	7.536 ± 0.122	220 ± 2	57 ± 2	2.2
SAL	5.684 ± 0.152	6.671 ± 0.501	93 ± 0	23 ± 0	3.0
SON	1.618 ± 0.086	3.019 ± 0.053	57 ± 1	23 ± 0	2.6
SAD	3.562 ± 0.177	7.3309 ± 0.085	72 ± 0	26 ± 1	2.9
DiOHBA	17.478 ± 0.503	20.133 ± 0.985	534 ± 1	373 ± 45	5.0
CAD	9.645 ± 0.416	2.058 ± 0.187	29 ± 0	36 ± 1	3.8
FAD	14.676 ± 1.691	3.592 ± 0.170	51 ± 7	14 ± 3	12.1
P/V[b]	1.04 ± 0.11	2.25 ± 0.04	1.39 ± 0.10	3.76 ± 0.28	6.7
S/V[c]	0.27 ± 0.01	1.11 ± 0.05	0.33 ± 0.03	0.48 ± 0.01	4.3
C/V[d]	0.60 ± 0.03	0.37 ± 0.01	0.12 ± 0.02	0.33 ± 0.01	6.6
$(Ad/Al)_v$[e]	0.87 ± 0.07	1.18 ± 0.01	0.73 ± 0.07	1.01 ± 0.01	5.0
$(Ad/Al)_s$[f]	0.63 ± 0.05	1.10 ± 0.07	0.77 ± 0.01	1.13 ± 0.07	5.3

[a] Errors are standard deviations (n = 3−5) or sample mean deviations (n = 2). [b] P = PAL + PON + PAD, V = VAL + VON + VAD. [c] S = SAL + SON + SAD. [d] C = CAD + FAD. [e] Ratio of VAD/VAL. [f] Ratio of SAD/SAL.

dx.doi.org/10.1021/ac202004r |Anal. Chem. 2012, 84, 459–464

that precipitates did not lead to any changes in recovery efficiencies if they were not removed before extraction. Precipitated inorganic particles in suspended sediment samples clogged the top frit of HLB cartridges, and they were removed by centrifugation before SPE.

The derivatization reaction of lignin phenols with BSTFA/TMS before chromatography was sensitive to acidic H-atoms. Traces of water and methanol in redissolved samples decreased reaction yields, and carboxylic acids showed lower yields than the corresponding aldehydes and ketones. Residual water in the HLB cartridge after air-drying was removed by elution through a cartridge packed with anhydrous sodium sulfate. Further, dry methanol was used for the second elution and samples were redissolved in dry pyridine.

Drying times for 2.5 mL of organic extract after elution were typically 3−4 h. We tested a Savant SVC200 vacuum centrifuge to speed-up solvent removal. Recovered concentrations after dry down at room temperature varied 8−45% (n = 4), indicating evaporative losses of internal standards and lignin phenols.

Method Performance. The precision and reproducibility of the method was examined by replicate analyses of DOM extracts and peat soil samples on different days (Table 3). Analyzed samples differed widely in concentrations and organic matter composition. Concentrations of phenols were reported in molar units instead of the traditional mass based units. Molar units are most appropriate for reporting molecular-level distributions of organic biomarkers such as lignin phenols.[17] At the nanomole level, relative standard deviations for individual lignin phenols were 1−12% (n = 3−5). At the picomole level, relative average deviations were 1−18% (n = 2). Relative errors for diagnostic lignin ratios did not exceed 16%. Analytical mean deviations of lignin phenols and calculated ratios averaged 4.6 ± 2.2%, overall (Table 3).

We also reanalyzed DOM extracts that were stored frozen at −20 °C for 7 years and had been analyzed by the traditional CuO oxidation procedure described by Hedges and Ertel[6] with modifications.[8] The analytical mean deviations averaged 11 ± 9% for all CuO oxidation products suggesting the method described here yields comparable results with the traditional CuO oxidation procedure (Table S-3 in the Supporting Information). Methanolic DOM extracts collected by reverse phase sorbents appeared to be stable for long periods of time (≤7 years) if stored at −20 °C.

CuO Oxidation Conditions. Yields of monomeric lignin phenols recovered after CuO oxidation are strongly affected by reaction temperature, additional oxidants such as oxygen, and sample size.[6,12]Previous studies have demonstrated the oxidation of phenolic aldehydes to corresponding carboxylic acids at elevated temperatures (>150 °C) or in the presence of molecular oxygen.[6,18] This side reaction also occurs in samples with an organic carbon content ≤2 mg of organic carbon.[12] The preferential removal of syringyl phenols and conversion of cinnamyl phenols to VAD and hydroxyl phenols has also been observed under conditions leading to superoxidation.[6,12,18] Glucose addition to samples with very low organic matter content was found to prevent oxidation of lignin phenol aldehyde groups to carboxyl groups and losses of syringyl and cinnamyl phenols.[12,19]

The effect of sample size on lignin phenol yields was tested with a DOM extract, peat soil, and suspended river sediment (Figure 2). Lignin phenol yields uniformly increased with sample size (Figure 2a,c,e), indicating oxidative losses of lignin phenols occurred at low organic matter content. The highest lignin phenol losses were observed with sample sizes <1.5 mg of OC

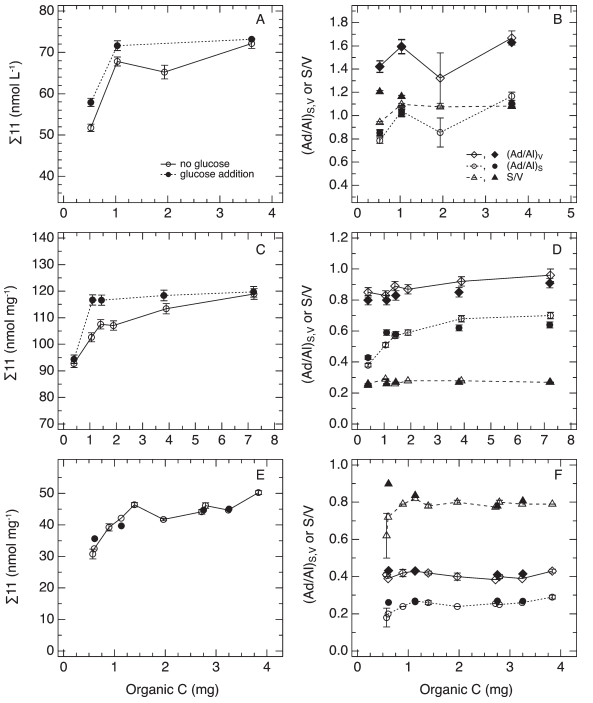


Figure 2. Lignin phenol concentrations (nmol L^{-1}or nmol mg^{-1}), acid/aldehyde ratios of vanillyl and syringyl phenols, and syringyl/vanillyl ratios in (A, B) DOM extract from the Colville River (Alaska), (C,D) peat soil, and (E, F) a sediment (wt % OC = 1.1). Solid symbols show analyses with glucose additions, whereas open symbols show analyses without glucose additions. Σ11 represents the sum of *p*-hydroxyl, syringyl, vanillyl, and cinnamyl phenols. Error bars show average mean deviations calculated from duplicate analyses of separate samples.

content, and lignin phenol yields were 8−39% lower than the highest yields measured. Above ∼1.5 mg of OC content, lignin phenol losses were less than 8% of the highest yields measured in the DOM extract and peat soil samples. The suspended river sediment showed relatively constant lignin phenol yields between 1.5 and 3.3 mg of OC content but increased by 12% at 3.8 mg of OC content. The current bomb design did not allow analysis of larger sediment samples. Glucose additions appeared to prevent losses of lignin phenols only in samples with ≥1 mg of OC content in the DOM extract and peat soil (Figure 2b,d). The effect of glucose was largely suppressed in suspended river sediment. This suggested the oxidation reaction was also sensitive to the sample matrix.

Syringyl and cinnamyl phenols were most sensitive to oxidation conditions, showing the largest relative losses at low organic carbon content (Figure 2b,d,f). The previously observed oxidation of aldehyde groups in lignin phenols to carboxyl groups at low organic carbon content did not occur under the current reaction conditions. Acid/aldehyde ratios of vanillyl and syringyl phenols were lowest at ≤1.5 mg of OC content. Adding glucose actually raised relative contributions of carboxylic acids at low organic carbon content in the river sediment but had minor effects in the DOM extract and soil sample. Acid/aldehyde ratios were not significantly different (t test, $p < 0.01$) in samples with >1.5 mg of OC.

In conclusion, the results suggest sample size should be carefully considered to prevent underestimation of lignin phenol concentrations. Glucose additions mitigate oxidation losses only to a certain extent. Using the CuO oxidation system described

dx.doi.org/10.1021/ac202004r |*Anal. Chem.* 2012, 84, 459–464

herein, we recommend samples contain at least 1.5 mg of OC and that all samples with <5 mg of OC be spiked with 10 mg of glucose as an additional carbon source. More tests need to be performed to clarify the suitability of the current analytical procedure for sediments with low organic carbon content.

■ ASSOCIATED CONTENT

Supporting Information. Figure S-1 and Tables S-1 and S-2. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

We thank Patrick Louchouarn and Rainer Amon for helpful discussions and sharing information on CuO oxidation procedures. This research was supported by National Science Foundation Grants 0843417 and 0850653.

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Supporting Information

Characterization of lignin by gas chromatography and mass spectrometry using a simplified CuO oxidation method

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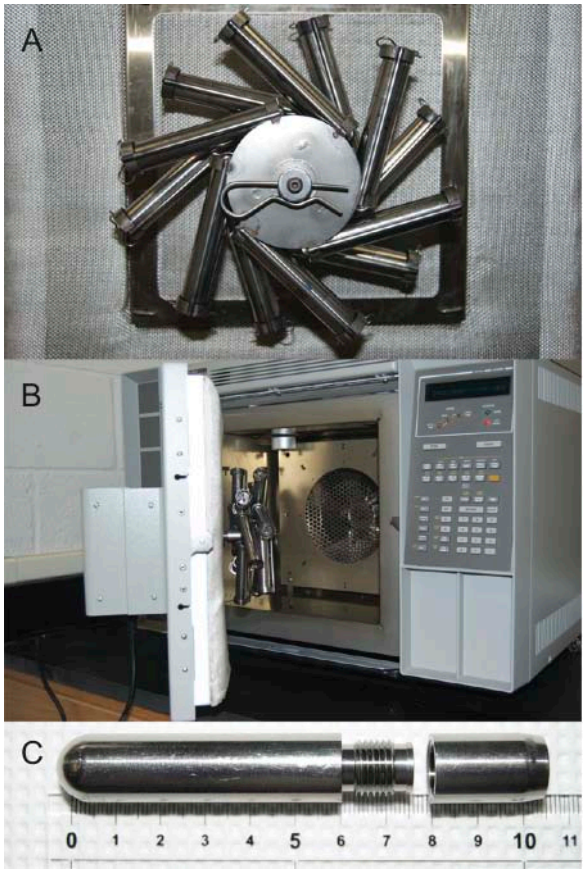

Figure S-1: (A, B) Ferris wheel type holder with reaction vessels mounted on the GC oven door as designed by the late John Hedges (University of Washington). An electric motor is mounted on the outside of the oven door. (C) Monel steel bomb with cap.

Table S-1: Method blank.

Compound	(pmol)[a]
CiAD	ND[b]
EVAL	ND
PAL	32±7
PON	22±3
PAD	70±7
VAL	54±14
VON	4±2
VAD	31±3
SAL	8±4
SON	1±0
SAD	2±1
DiOHBA	795±111
CAD	3±1
FAD	7±0

[a] Sample standard deviations for the method blank were calculated from 6 replicates measured on different days.
[b] ND, not detected.

Table S-2: Recoveries of lignin phenols using one elution with chloroform/methylacetate/pyridine.

	Elution: 1.5 mL Ch_2Cl_2/MeAc/Pyr (75/25/5 v/v%)	
	(n=4)	(n=4)
Compound	(%)	Relative to CiAD (%)
CiAD	86.4±3.2	100.0
EVAL	85.9±3.0	99.4±0.7
PAL	84.4±3.9	97.6±1.3
PON	82.3±4.0	95.2±1.2
PAD	76.4±6.1	88.4±6.0
VAL	84.0±4.2	97.2±1.2
VON	86.1±3.8	99.6±1.6
VAD	80.0±3.5	92.6±2.4
SAL	85.6±5.2	99.0±2.4
SON	85.5±4.3	98.9±2.2
SAD	77.5±4.8	89.6±3.1
DiOHBA	<10	NC[a]
CAD	84.8±4.0	98.1±1.8
FAD	83.1±8.1	96.0±5.9

[a] NC, not calculated

Table S-3: Comparison of CuO oxidation products obtained with the described method versus the old published method. Values express percent mean deviations (n=2).

	Method comparison[a]	
	Gate Creek River[b]	Arctic Ocean surface[c]
Compound	River	surface
PAL	7.0	2.5
PON	4.3	1.3
PAD	3.7	1.8
VAL	1.2	4.8
VON	1.1	9.4
VAD	9.5	13.9
SAL	11.6	15.5
SON	4.4	7.9
SAD	26.1	24.2
DiOHBA	11.5	12.3
CAD	10.2	17.3
FAD	23.7	38.9
$\sum 8$	3.7	3.5
$\sum 6$	7.1	8.7
P/V	8.2	3.4
S/V	11.4	11.4
C/V	13.8	24.4
$(Ac/Al)_v$	10.7	18.5
$(Ac/Al)_s$	14.9	9.0

[a]Sample analyses using the old method were performed in 2004 according to Hedges and Ertel (1982) with modifications described by Opsahl and Benner (1997) and Louchouarn et al. (2000). Samples were stored in methanol for 7 years at -20 °C until reanalysis.
[b] Concentrations of phenols ranged from 31-201 nmol L^{-1}
[c] Concentrations of phenols ranged from 83-964 pmol L^{-1}