Application of Chloride Adduct Ionization Tandem Mass Spectrometry for Characterizing and Sequencing Synthetic Lignin **Model Compounds**

Shardrack O. Asare, Poorya Kamali, Fan Huang, and Bert C. Lynn*

Department of Chemistry, University of Kentucky, Lexington, Kentucky 40506, United States

Supporting Information

ABSTRACT: The need for renewable bioenergy sources has renewed interest in lignin chemistry; however, structural elucidation and characterization of lignin degradation products remain a challenge because of lack of effective analytical methods. The analysis of lignin oligomers has been accomplished by simple deprotonation of weakly acidic phenolic moieties using NaOH and analyzed in a negative ESI mass spectrometry. Although simple deprotonation works to produce excellent results for many types of lignin compounds, others can undergo extensive in-source fragmentation for certain bond types making structural elucidation more complicated. Herein, we present an alternative method for analyzing lignin model compounds using chloride adduct chemistry. In this study, nine β -O-4 dimers, an (4-O- $\alpha)(\beta$ -O-4) trimer, and a $(\beta$ -O-4)(β -O-4) trimer were synthesized and analyzed using chloride adduct mass spectrometry in the negative mode using NH₄Cl as the chloride source. Stable chloride adducted molecular ions were observed for all analyzed compounds. Tandem mass spectrometry experiments performed on each precursor ion produced "signature" fragment ions specific to each analyte. The compelling features of this method include the production of stable chloride adduct molecular ions that do not undergo in-source fragmentation, in contrast to simple deprotonation methods that can lead to extensive fragmentation for some structures, the appearance of the chlorine isotope pattern for enhanced recognition of molecular ions, and production of monolignol sequence specific fragment ions using tandem mass spectrometry.

1. INTRODUCTION

The need for renewable bioenergy sources has renewed interest in lignin chemistry. Lignin is the second most abundant polymer present in the plant cell wall and is composed of three phenylpropanoid monolignol monomers: 4-hydroxycinnamyl alcohol, coniferyl alcohol, and sinapyl alcohol. Lignin has the potential of becoming the world's alternative source for biobased synthons in fine chemicals, polymers and pharmaceuticals. 1-5 Despite the promising applications of lignin, structural elucidation and characterization of lignin degradation products remain a challenge because of lack of effective analytical methods. 6-11

Mass spectrometry is a powerful analytical technique that has been proven to be useful in the characterization of complex mixtures without the need for time-consuming isolation and purification, compared to other methods like nuclear magnetic resonance. 12,13 Even though mass spectrometry can provide molecular weight and elemental compositions using high-resolution instruments, its application in lignin studies has remained a challenge due to the poor ionization efficiency of lignin and lignin degradation products. 13-15 Lignin degradation products are mainly composed of complex mixtures that are difficult to characterize without purification and hence mass spectral analysis of lignin degradation products will require an efficient ionization process that will enable the ionization of all components in the mixture without greatly increasing the internal energy of the ion and subsequent fragmentation. 16,17 Mild but efficient ionization is essential to limit the complexity of the resulting mass spectrum where only one ion is generated for each analyte.

Electrospray combines desorption and ionization into a means of effectively analyzing lignin. Because electrospray is dominated by solution chemistry, solution phase analyte ions in aerosol droplets are effectively transported into gas phase analyte ions during the electrospray process. 18 The most common ionization in electrospray involves simple protonation. 19 Unfortunately, no evidence exists for lignin compounds accepting protons regardless of solution pH. However, cationization by ubiquitous sodium and potassium ions is readily observed under these circumstances. 19 Haupert et al. utilized electrospray ionization using methanol/water as solvent in a positive and negative ion mode to characterize lignin model compounds. For the five model compounds analyzed, they observed a poor ion signal for several of the analytes, which suggested that only molecules with weakly acidic phenolic moieties were ionized.²⁰ This ionization method proved not to be suitable for the analysis of complex lignin degradation products. On the basis of on these observations, it would appear that positive ion mass spectrometry of lignin and lignin degradation products would best be served by nonproton, hard cationization methods.

Negative ion electrospray is typically dominated by simple deprotonation of acidic analytes ([M-H⁺]⁻). Because most lignin and lignin degradation products contain relatively acidic free phenol moieties, simple deprotonation negative ionization electrospray should be effective. Haupert et al. recently developed an electrospray/tandem mass spectrometry method for the characterization of lignin model compounds via simple deprotonation.²⁰ They showed that by doping a mixture of lignin model

Received: February 12, 2018 Revised: March 30, 2018 Published: April 8, 2018



$$R^1$$
 A
 OH
 R^3
 OH
 B
 OH
 B

$$\begin{split} R^1 = & R^2 = R^3 = R^4 = H, \ H - (\beta - O - 4) - H, \ R^1 = R^2 = R^3 = H, \ R^4 = O C H_3, \ H - (\beta - O - 4) - G, \ R^1 = R^2 = H, \\ R^3 = & R^4 = O C H_3, \ H - (\beta - O - 4) - S, \ R^1 = O C H_3, \ R^2 = R^3 = R^4 = H, \ G - (\beta - O - 4) - H, \ R^1 = H, \ R^2 = O C H_3, \ R^3 = R^4 = O C H_3, \ G - (\beta - O - 4) - S, \ R^1 = R^2 = O C H_3, \\ R^3 = & R^4 = H, \ S - (\beta - O - 4) - H, \ R^1 = R^2 = O C H_3, \ R^3 = O C H_3, \ R^4 = H, \ S - (\beta - O - 4) - G, \ R^1 = R^2 = R^3 = R^4 = O C H_3, \ S - (\beta - O - 4) - S. \end{split}$$

H-(
$$\beta$$
-O-4)-G-(β -O-4)-S G-(4-O- α)-G-(β -O-4)-G

Figure 1. Synthesized lignin model compounds.

compounds with a basic solution, a single deprotonated molecular ion per analyte was observed, which upon isolation and tandem mass spectrometry provided useful structural information for their studied model compounds.

Recently, we synthesized, isolated, and characterized three lignin model trimers and tested the simple deprotonation electrospray mass spectrometry of these compounds.²¹ Two of the three behaved as expected and produced abundant [M-H+]ions. However, the third trimer, a $G(4-O-\alpha)G(\beta-O-4)G$ (Figure 1) trimer responded poorly. The mass spectrum of the G(4-O- α)G(β -O-4)G trimer showed very abundant m/z 357 and m/z179 ions (consistent with the mass-to-charge ratio of dimer and monomer, respectively) and a relatively weak molecular ion at m/z 537. Because all of the trimers were pure isolates, these lower mass ions were thought to result from extensive in-source fragmentation of $G(4-O-\alpha)G(\beta-O-4)G$. Comparing the structural difference in three trimers, we hypothesized that G(4-O- α)G(β -O-4)G trimer undergoes a charge-driven in-source fragmentation pathway initiated by the deprotonation step. While low in abundance, $(4 - O - \alpha)$ bonds have been observed in natural lignin. 23 Using simple deprotonation electrospray, these bonding motifs would be missed. More importantly, the presence of these $(4-O-\alpha)$ bonds would greatly increase the confusion in spectra from deprotonated molecular ions as a result of their in-source fragmentation.

Here, we present an alternative mass spectrometric method of ionizing lignin model compounds that may be part of lignin degradation products. This negative ion electrospray method utilizes

ammonium chloride^{24,25} as a dopant that produces stable chloride adducts of the precursor ions, [M+Cl⁻]⁻. In this work, we demonstrated that chloride adduct ionization is an alternative ionization method that efficiently produces stable chloride adductmolecular ions for each analyte, an essential feature for determining the molecular weight of an unknown compounds and furthermore these chloride adducted molecular ions produce an unambiguous sequence specific fragmentation for each molecule studied. Nine lignin model dimers containing a β -0-4 bond, an all β -0-4 trimer and a mixed α,β trimer were synthesized, and analyzed as their chloride adducts using two mass spectrometer systems, a linear quadrupole ion trap mass spectrometer (LTQ) and a highresolution accurate-mass Q Exactive orbitrap mass spectrometer (Q Exactive). Structural relevant information and sequencing were obtained by performing collision-induced dissociation (CID) and high energy collision dissociation (HCD) tandem mass spectrometry on each isolated ion of interest.

2. MATERIALS AND METHODS

2.1. Methods. The synthesis of G-(4-O- α)-G-(β -O-4)-G has been reported previously. Nine lignin dimer model compounds, H-(β -O-4)-H, H-(β -O-4)-G, H-(β -O-4)-S, G-(β -O-4)-H, G-(β -O-4)-G, G-(β -O-4)-S, S-(β -O-4)-H, S-(β -O-4)-G, S-(β -O-4)-S, and a trimer H-(β -O-4)-G-(β -O-4)-S were synthesized using published protocol with slight modification. Picely 3 Briefly, dimers were prepared by protection, aldol coupling, reduction, and deprotection reactions (details can be found in the Supporting Information).

2.2. Mass Spectrometry Experiments. All experiments were carried out using a ThermoScientific Q Exactive orbitrap mass

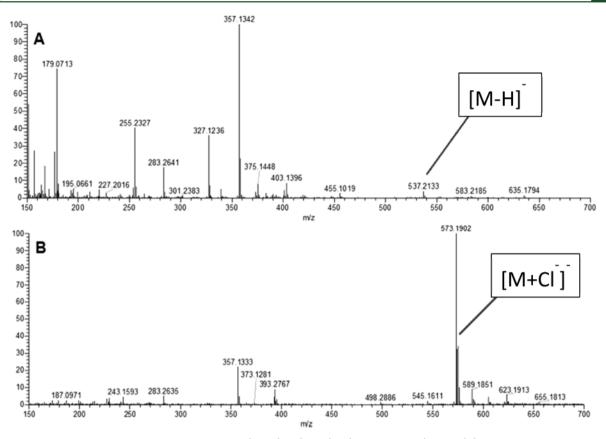


Figure 2. Q Exactive negative ion mode full mass spectrum of $G-(4-O-\alpha)-G-(\beta-O-4)-G$ (MW- 538 g/mol) trimer. (A) Spectrum of a deprotonated $G-(4-O-\alpha)-G-(\beta-O-4)-G$ (m/z 537) indicating the extensive in-source fragmentation. (B) Spectrum of a chloride adduct of $G-(4-O-\alpha)-G-(\beta-O-4)-G$ (m/z 573).

spectrometer equipped with an HESI source and a ThermoScientific LTQ linear ion trap mass spectrometer equipped with an ESI source. The ESI conditions were 4–4.5 kV spray voltage, nitrogen sheath and auxiliary gas flow of 22 and 12 (arbitrary units Q Exactive and LTQ, respectively) and mass spectrometer inlets temperature of 250 °C. Data were acquired using the ThermoScientific Xcalibur software in the tune mode.

Stock solutions for all studied model compounds were prepared to a final concentration of 1 mM in acetonitrile. For specific direct infusion experiments, an aliquot of the stock solution was diluted with an equal volume of aqueous ammonium chloride solution to produce 50% ACN:50% aqueous ammonium chloride solution that was introduced into the mass spectrometer with a syringe pump at a flow rate of 3uL per minute.

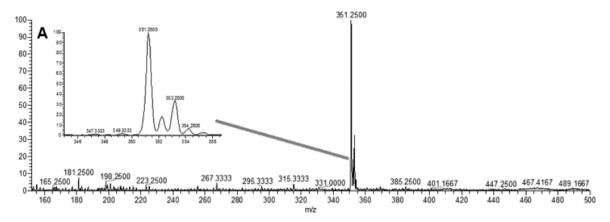
For LTQ collision-induced dissociation (CID) experiments, the mass spectrometer was operated in advance scan mode. The desired precursor ion was isolated using an m/z window of 3 units and a q value of 0.25. Selected ions were dissociated with a percent normalized collision energy (%NCE) between 0 and 35% NCE with helium as the collision gas for an activation time of 25 ms. In high energy collision-dissociation (HCD) experiments, the Q Exactive mass spectrometer was operated in the advance scan mode and was used to isolate desired ions using an m/z window of 3 units. Selected ions were subjected to a HCD voltage with a normalized collision energy of 18 using nitrogen as the collision gas. The Q Exactive mass spectra were obtained at a mass resolution of 140 000.

3. RESULTS AND DISCUSSION

Nine model monolignol dimers and two trimers, $G-(4 O-\alpha)-G-(\beta-O-4)-G$ and $H-(\beta-O-4)-G-(\beta-O-4)-S$ were analyzed using the chloride adduct mass spectrometric method. Structural information based on (-) CID/MS³ and (-) HCD/MS/MS were obtained with a linear ion trap mass spectrometer and a Q Exactive orbitrap mass spectrometer and the results of the two experiments are discussed below. Initial experiments determined

that the minimum concentration of NH₄Cl required for efficient adduct formation was 0.1 mM and hence all experiments were done using this concentration.

3.1. Full Mass Spectra of Studied Compounds. In an attempt to improve existing analytical methods for analyzing lignin degradation products, we implemented a chloride adduct method^{28,29} to analyze lignin. As already stated, efficient generation of analyte ions without subsequent fragmentation is crucial to mass spectrometry measurements and hence it is important that the chosen method can effectively ionize the analyte without causing fragmentations. Figure 2a shows a full scan mass spectrum of a G-(4-O- α)-G-(β -O-4)-G trimer analyzed by simple deprotonation. Substantial in-source fragmentation was observed for the deprotonated analyte which suggests that mass spectral analysis of these type of lignin compounds may not be very efficient. However, Figure 2b shows the mass spectrum of the chloride adduct of the same G-(4-O- α)-G-(β -O-4)-G trimer where virtually no in-source fragmentation was observed. This method of ionization was extended to other model compounds. As an example, a H-(β -O-4)-H (MW- 316 g/mol) dimer was analyzed and the chloride adduct was the base peak (Figure 3a). A similar result was also obtained for the trimer H-(β -O-4)-G-(β -O-4)-S when analyzed as a chloride adduct. (Figure 3b). It is worth noting that chloride has two isotopes ³⁵Cl and ³⁷Cl, with a 3:1 relative abundance. Both isotopes were observed for the chloride adduct in the full spectrum. This represents an added advantage of chloride adduct mass spectrometry for confirmation purposes. This observation was consistent across all molecules studied. It has been reported that lignin easily forms a sodium adduct in positive ion mode because of the trace amount of sodium in most solutions.²⁰ Therefore, we used the sodium



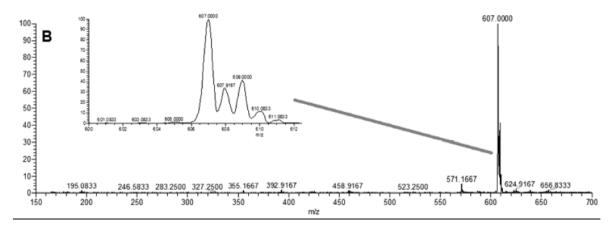


Figure 3. (A) Full scan LTQ spectra of H-(β -O-4)-H (316 g/mol, [M+Cl]⁻ m/z 351) dimer and (B) H-(β -O-4)-G-(β -O-4)-S (572 g/mol, [M+Cl]⁻ m/z 607) trimer. The inset shows the mass region around the chloride adduct molecular ion highlighting the isotope pattern.

adduct-molecular ions to determine the accurate mass for all the compounds studied. The accurate mass results for Na $^+$ adducts are shown in Table S1. Data from chloride attachment spectra are shown in Table 1 as the exact mass with their corresponding error for all nine dimers. Errors observed for exact mass measurement were within an acceptable range of (\pm) 3.5 ppm.

3.2. (-) CID/MS³ Structural Studies. To study the structural information that could be obtained from the chloride adduct analysis, all nine model dimers, H-(β -O-4)-H (MW- 316 g/mol), $H-(\beta-O-4)-G$ (MW- 346 g/mol), $H-(\beta-O-4)-S$ (MW- 376 g/mol), G-(β -O-4)-H (MW- 346 g/mol), G-(β -O-4)-G (MW- 376 g/mol), G-(β -O-4)-S (MW- 406 g/mol), S-(β -O-4)-H (MW- 376 g/mol), $S-(\beta-O-4)-G$ (MW- 406 g/mol), $S-(\beta-O-4)-S$ (MW- 436 g/mol), and H-(β -O-4)-G-(β -O-4)-S (MW- 572 g/mol), were made in acetonitrile and doped with ammonium chloride solution and infused directly into the mass spectrometer and a spectrum recorded in the negative ion mode. A stable chloride adduct was formed for each molecule with one product ion per molecule without in-source fragmentation, this observation clearly suggests that ammonium chloride doped ionization process is an effective alternative way of ionizing lignin degradation product. Since lignin degradation products typically consist of complex mixtures of different products with different compositions, mixture analysis becomes important. For example, if one attempts to analyze a mixture containing all nine lignin dimers, ions at m/z 381, m/z 411, and m/z 441 will have higher intensities because these ions represent empirical formula overlaps in the dimer series. Therefore, tandem mass spectral structural information would be required to differentiate the overlapping molecular ions. On the LTQ linear ion trap, a typical experiment would involve isolation of the chloride adduct molecular ion as the precursor and CID at 15% NCE. The resulting MS² spectrum would show formation of [M-H]⁻ as the exclusive product ion from the loss of HCl. Since the [M-H]⁻ ion was formed in the ion trap and collisionally cooled with helium, this ion shows remarkable stability as evidenced by storage in the ion trap for 10 s. Application of the MS³ experiment at 15% NCE showed characteristic product ions consistent with the monolignol sequence of the model compounds. For example, the MS² spectrum of H-(β -O-4)-G showed selective conversion of m/z 381 to m/z 345 ([M-H]⁻) (Figure 4a). The MS³ spectrum showed three significant product ions m/z 297, m/z 179 and m/z 165 where the ions at m/z 165 and m/z 179 are specific to the H-(β -O-4)-G sequence (Figure 4b).

The chloride adduct molecular ion of all nine dimers showed a conserved MS^3 fragmentation pattern composed of two fragmentation pathways. The dominant pathway showed a loss of 48 mass units that corresponded to the loss of water and formaldehyde from $[M-H]^-$ generated in the MS^2 experiment. The second channel that involved breaking the β -0–4 bond to give "signature" sequence specific fragment ions.

For comparison, H-(β -O-4)-S, S-(β -O-4)-H, and G-(β -O-4)-G dimers produced the same molecular ion at m/z 411 [M+Cl]⁻ in the full spectrum because these molecules have the same molar mass (see Figure 5). The MS² spectra for all three dimers are virtually identical and showed selective conversion to the m/z 375 [M-H]⁻ product ion, which does not provide structural information. The MS³ spectrum of H-(β -O-4)-S (m/z 411)

Table 1. Accurate Mass Data for All Nine (β -O-4) Dimers Analyzed As the Chloride Adduct

Name	Structure	MW	Calc. m/z [M+Cl]	Obs. m/z [M+Cl]	Error (ppm)
Η-(β-Ο-4)-Η	ОН	316	351.1005	351.0991	-3.94
Η-(β-Ο-4)-G	он о он	346	381.111	381.1097	-3.48
Η-(β-Ο-4)-S	но он о он	376	411.1216	411.12	-3.99
G-(β-O-4)-H	но он он	346	381.1101	381.1099	-3.08
G-(β-O-4)-G	но но о	376	411.1216	411.1201	-3.61
G-(β-O-4)-S	HO HO O OH	406	441.1322	441.1307	-3.31
S-(β-Ο-4)-Η	HO OHO OH	376	411.1216	411.1201	-3.69
S-(β-O-4)-G	HO HO OH	406	441.1322	441.1308	-3.1
S-(β-O-4)-S	НО НО ОН	436	471.1427	471.1411	-3.37

showed a base peak at m/z 327 that resulted from the loss of H_2O and CH₂O (48 amu) from the [M-H]⁻ precursor ion. This result is consistent with previous studies^{22,30} which suggested a loss of hydroxyl group at the α position and loss of hydroxymethyl group at the gamma positon of the β -O-4 linkage of a guaiacylglycerol-beta-guaiacyl ether dimer. More importantly, the MS³ spectrum of H-(β -O-4)-S also showed unique fragment ions at m/z 165 and m/z 209 indicative of the HS sequence. In contrast, MS³ spectrum of S-(β -O-4)-H (the sequence isomer of H-(β -O-4)-S) showed unique products ions at m/z 149 and m/z 225 clearly differentiating SH from its sequence isomer, HS. Additionally, the MS³ spectrum of G-(β -O-4)-G dimer showed sequence specific product ions at m/z 179 and m/z 195 and these fragment ions corresponds to "ring A" (m/z 195) and "ring B" (m/z 179). Similar fragmentation patterns were also observed for other sequence isomers (Table 2). Similar sequence specific fragment ions were recently reported by Sheng et al.31 This distinct difference in the MS3 mass spectrum allows for the unambiguous identification and differentiation of products having the same empirical formula but different sequences. This observation establishes the usefulness of chloride adduct ionization coupled to tandem mass spectrometry in the negative ion mode for the characterization of an unknown lignin compounds.

Sequence-specific information was also obtained for the H-(β -O-4)-G-(β -O-4)-S (MW = 572 g/mol) trimer. A stable chloride adduct molecular ion was observed at m/z 607 (Figure 3b) in the full scan spectrum. The MS³ spectrum (m/z 607 to m/z 571 to products) showed dominant product ions at m/z 523, m/z 405, and m/z 361, three monolignol specific ions at m/z 165, m/z 195, and m/z 209 corresponding to ring A, ring B, and ring C, respectively. The m/z 523 ion resulted from the loss of 48 amu [H₂O-CH₂O] from [M-H] $^-$. Cleavage of the ring A/ring B bond resulted in the formation of dimer fragment at m/z 405 corresponding to G-(β -O-4)-S dimer anion. Likewise, the cleavage of the ring B/ring C bond resulted in a second dimer fragment at m/z 361 corresponding to H-(β -O-4)-G dimer

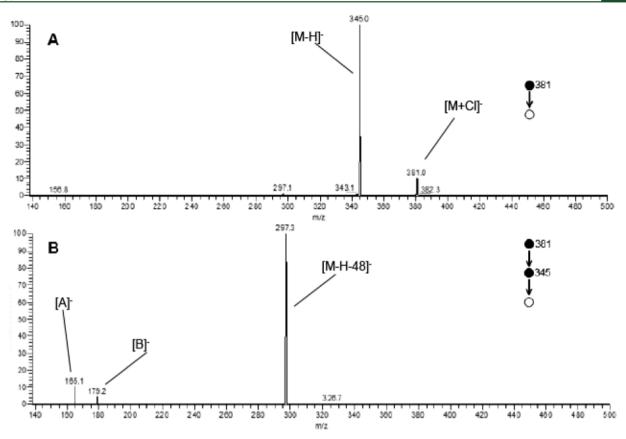


Figure 4. LTQ (-) CID spectra of H-(β-O-4)-G (346 g/mol) dimer where A shows the MS² spectrum and B shows the MS³ spectrum.

anion. This m/z 361 H-(β -O-4)-G anion loses [H₂O-CH₂O] to produce m/z 313. Thus, the observed monolignol fragments in concert with the dimer ions can be used to uniquely identify the sequence of this H-(β -O-4)-G-(β -O-4)-S trimer. Table S2 shows the fragment ions observed for H-(β -O-4)-G-(β -O-4)-S trimer.

3.3. (–) HCD/MS/MS Structural Studies. To study the robustness of this ionization method, high-energy collisional dissociation (HCD) spectra were obtained for all of the model compounds studied using the Q Exactive. Interestingly, we observed that a simple tandem mass spectrometry using HCD was sufficient to provide useful structural information and near identical sequence-specific data compared to the LTQ CID/MS³ experiments. Figure S7 (Supporting Information) shows the HCD MS² spectrum for H-(β -O-4)-H dimer as the chloride adduct (m/z 351). Because of the increased collisional energy, nitrogen as a collision gas and increased probability of multiple collisions, the HCD MS² spectrum shows the same characteristics fragment ion as observed in the MS³ LTQ experiment.

We compared the HCD MS² mass spectra of the chloride molecular ions from H-(β -O-4)-G-(β -O-4)-S trimer (Figure 6a) with the G-(4-O- α)-G-(β -O-4)-G trimer (Figure 6b). The G-(4-O- α)-G-(β -O-4)-G spectrum was quite simple compared to the all (β -O-4) trimer. The $\alpha\beta$ -all G trimer spectrum was dominated by an intense m/z 179 corresponding to both ring A and ring C. The m/z 357 ion likely resulted from cleavage of the (4-O- α) bond with the resulting dimer carrying the charge.

3.4. (–) CID/MS³ Energy Titration. A titration experiment was done to determine the efficiency and optimum mass spectrometric conditions for the analysis of lignin model compounds related to the lignome using ammonium chloride as a dopant.

In this experiment, the chloride adduct molecular ion was isolated in the linear ion trap mass analyzer, an increasing CID voltage was then applied and a spectrum was recorded. In a typical titration experiment, [M-H]⁻ from the MS² experiment was isolated at a 0% NCE in the LTQ, the CID voltage was then increased in increments of 5 from 0% to 35% NCE and the mass spectrum recorded. The observed fragment ions in the MS³ had different relative abundance depending on the structure of the molecule. We observed that at 15% CID voltage, the chloride is lost from the molecular ion as HCl, which lead to the formation of a stable [M-H] with a 100% relative intensity. An initial experiment determined that the appropriate CID voltage for tandem analysis was 15% NCE, all nine dimers followed similar fragmentation patterns with varying relative abundance of fragment ions. Table 2 shows fragment ions and abundance at 15% NCE. Additionally, each of the nine dimers fragmented at the β -O-4 link to give a signature "ring A" and "ring B" of each molecule. It must be noted that the relative abundance of fragment ions (ring A and ring B) varied significantly depending on the composition of the dimer. This structure- based effect on the abundance of each fragment ion was evaluated. In general, dimers with no methoxy group on the "ring B" produced lowintensity fragment ions (see Table 2). Addition of one methoxy group on the "ring B" increased the relative abundance of the fragment ions. Molecules having two methoxy groups on the "ring B" had the highest relative intensities in term of fragment ions. Although the reason for this trend is not completely known, we speculate that having the methoxy group on the "ring B" increases the electron density and hence promotes facile cleavage of the β -O-4 bond. An in-depth mechanistic and structuredependent fragmentation studies are currently underway and will be reported separately.

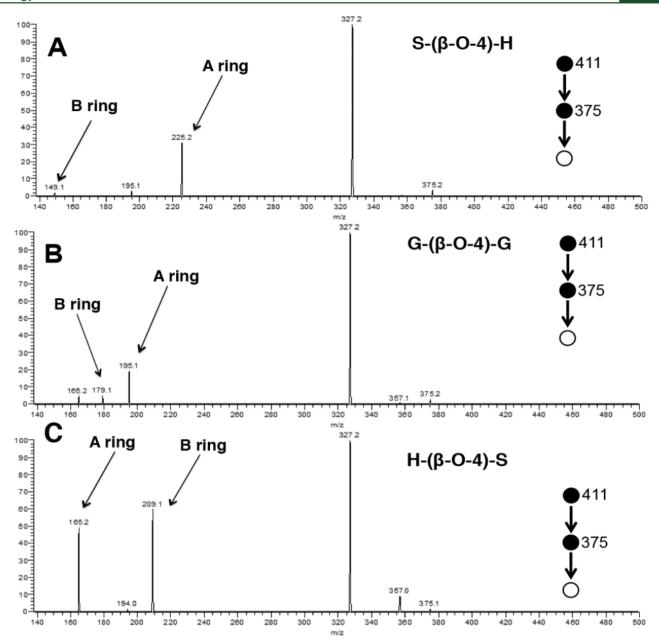


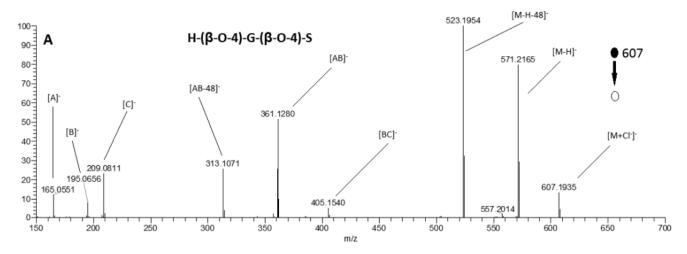
Figure 5. LTQ (-) CID/MS³ spectra of (A) S-(β -O-4)-H (m/z 411), (B) G-(β -O-4)-G (m/z 411), and (C) H-(β -O-4)-S (m/z 411) analyzed as a chloride adduct.

Table 2. MS³ of Diagnostic Fragment Ions at 15% NCE

name	MS ³ CID 15%					
	[M-H] ⁻ (%)	$[M-H_2O-CH_2O]^-$ (%)	ring A (%)	ring B (%)		
H-(β-O-4)-H	m/z 315 (12.79)	m/z 267 (59.39)	m/z 165 (6.93)	m/z 149 (4.29)		
H-(β-O-4)-G	m/z 345 (9.56)	m/z 297 (62.68)	m/z 165 (8.02)	m/z 179 (3.61)		
H-(β-O-4)-S	m/z 375 (1.17)	m/z 327 (37.99)	m/z 165 (25.01)	m/z 209 (17.87)		
G-(β-O-4)-H	m/z 345 (20.14)	m/z 297 (52.46)	m/z 195 (9.73)	m/z 149 (1.76)		
G-(β-O-4)-G	m/z 375 (7.98)	m/z 327 (52.30)	m/z 195 (4.32)	m/z 179 (3.01)		
G-(β-O-4)-S	m/z 405 (2.44)	m/z 357 (28.99)	m/z195 (25.07)	m/z 209 (20.61)		
S-(β-O-4)-H	m/z 375 (2.19)	m/z 327 (63.46)	m/z 225 (20.71)	m/z 149 (1.04)		
S-(β-O-4)-G	m/z 405 (3.68)	m/z 357 (53.41)	m/z 225 (17.78)	m/z 179 (2.54)		
S-(β-O-4)-S	m/z 435 (1.03)	m/z 387 (26.45)	m/z 225 (37.19)	m/z 209 (9.60)		

4. CONCLUSION

Mass spectrometry is a powerful analytical technique used in structural elucidation of unknown compounds. It recently gained popularity in the analysis of lignin degradation products because of its ability to provide an exact mass of a molecule and fast analysis time. To date, the best way to gain efficient ionization of



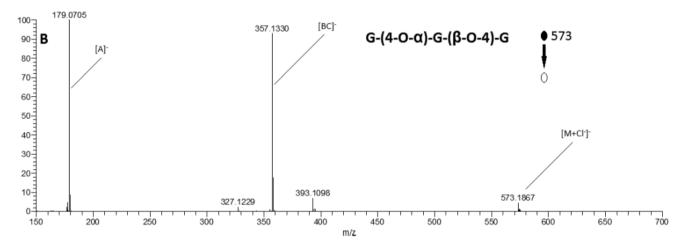


Figure 6. (A) Q Exactive (-) HCD/MS² spectrum of H-(β -O-4)-G-(β -O-4)-S trimer and (B) Q Exactive (-) HCD/MS² spectrum G-(4-O- α)-G-(β -O-4)-G trimer.

lignin-related compounds is by simple deprotonation using NaOH. Although simple deprotonation works to produce excellent results for many types of lignin compounds, others can undergo extensive in-source fragmentation thus making structural elucidation more complicated. We have successfully proven that chloride adduct formation is capable of producing stable molecular ions which are essential in accurate mass determination. More importantly, tandem mass spectrometry of chloride adduct molecular ions provides unambiguous sequence specific fragment ions which enables the identification and characterization of dimers and trimers. Additionally, the recommended concentration of ammonium chloride dopant of 0.1 mM has little impact on the long-term operation of the mass spectrometer.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.energyfuels.8b00559.

Synthesis details, Figures S1–S7, Schemes S1 and S2, and Tables S1 and S2 (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: bclynn2@uky.edu. Phone: 859-218-6529, Fax: 859-257-2489. Web site: http://www.research.uky.edu/ukmsf.

ORCID ®

Bert C. Lynn: 0000-0001-8426-3024

Present Address

[†]F.H. is currently at Solenis, LLC, 500 Hercules Road, Wilmington, DE 19808

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Authors S.A., P.K., and B.C.L. acknowledge the National Science Foundation EPSCoR Track 2 (OIA 1632854) for financial support.

REFERENCES

- (1) Boerjan, W.; Ralph, J.; Baucher, M. Annu. Rev. Plant Biol. 2003, 54, 519-546.
- (2) Alejandro, S.; Lee, Y.; Tohge, T.; Sudre, D.; Osorio, S.; Park, J.; Bovet, L.; Lee, Y.; Geldner, N.; Fernie, A. R.; Martinoia, E. *Curr. Biol.* 2012, 22, 1207–1212.
- (3) Ragauskas, A. J.; Beckham, G. T.; Biddy, M. J.; Chandra, R.; Chen, F.; Davis, M. F.; Davison, B. H.; Dixon, R. A.; Gilna, P.; Keller, M.; et al. *Science* 2014, 344, 1256843.
- (4) Eudes, A.; Liang, Y.; Mitra, P.; Loque, D. Curr. Opin. Biotechnol. 2014, 26, 189-198.
- (5) Kosyakov, D. S.; Ul'yanovskii, N. V.; Anikeenko, E. A.; Gorbova, S. N. Rapid Commun. Mass Spectrom. 2016, 30 (19), 2099–2108.

(6) Doherty, W. O. S.; Mousavioun, P.; Fellows, C. M. *Ind. Crops Prod.* 2011, 33, 259–276.

- (7) Saito, K.; Kato, T.; Tsuji, Y.; Fukushima, F. Biomacromolecules 2005, 6, 678-683.
- (8) Costell, E. C.; Contado-Miller, M. J.; Cipollo, F. J. J. Am. Soc. Mass Spectrom. 2007, 18, 1799-1807.
- (9) Zakzeski, J.; Bruijnincx, P. C. A.; Jongerius, A. L.; Weckhuysen, B. M. Chem. Rev. 2010, 110, 3552–3599.
- (10) Boerjan, W.; Ralph, J.; Baucher, M. Annu. Rev. Plant Biol. 2003, 54, 519-546.
- (11) Cherubini, F.; Stromman, A. H. Energy Fuels 2010, 24, 2657–2666.
- (12) Cooks, R.; Busch, K.; Glish, G. Science 1983, 222, 273-291.
- (13) McLafferty, F. W. Int. J. Mass Spectrom. 2001, 212, 81-87.
- (14) Amy, J. W.; Baitinger, W. E.; Cooks, R. G. J. Am. Soc. Mass Spectrom. 1990, 1, 119-128.
- (15) de Hoffmann, E.; Stroobant, V. Mass Spectrometry: Principles and Applications; Wiley: New York, 2007.
- (16) Watson, J. T.; Sparkman, O. D. Introduction to Mass Spectrometry: Instrumentation, Applications and Data Interpretation; Wiley & Sons: West Sussex, U.K., 2008.
- (17) Skoog, D. A.; Holler, F. J.; Crouch, S. R. Principles of Instrumental Analysis: Brooks ColeBelmont, CA. 2007.
- (18) Smith, E. A.; Park, S.; Klein, A. T.; Lee, Y. J. Energy Fuels 2012, 26, 3796-3802.
- (19) Cheng, S. C.; Cheng, T. L.; Chang, H. C.; Shiea, J. Anal. Chem. 2009, 81, 868-74.
- (20) Haupert, L. J.; Owen, B. C.; Marcum, C. L.; Jarrell, T. M.; Pulliam, C. J.; Amundson, L. M.; Narra, P.; Aqueel, M. S.; Parsell, T. H.; Abu-Omar, M. M.; Kenttamaa, I. H. Fuel 2012, 95, 634–641.
- (21) Huang, F. Application of High-Resolution Accurate Mass (HRAM) Mass Spectrometry for Analysis of Lignin Model Compounds and the Post-Pretreatment Products. *Ph.D. dissertation*; University of Kentucky, Lexington, KY, 2017.
- (22) Morreel, K.; Kim, H.; Lu, F.; Dima, O.; Akiyama, T.; Vanholme, R.; Niculaes, C.; Goeminne, G.; Inze, D.; Messens, E.; Ralph, J.; Boerjan, W. *Anal. Chem.* 2010, 82, 8095–8105.
- (23) Banoub, J.; Delmas, H. G.; Joly, N.; Mackenzie, G.; Cachet, N.; Benjelloun-Mlayah, B.; Delmas, M. J. Mass Spectrom. 2015, 50, 5–48.
- (24) Vinueza, N. R.; Gallardo, V A.; Klimek, J. F.; Carpita, N. C.; Kenttämaa, H. I. Fuel 2013, 105, 235–246.
- (25) Alsbou, E.; Helleur, B. Energy Fuels 2014, 28, 578-590.
- (26) Katahira, R.; Kamitakahara, H.; Takano, T.; Nakatsubo, F. J. Wood Sci. 2006, 52, 255–260.
- (27) Chu, S.; Subrahmanyam, A. V.; Huber, G. W. Green Chem. 2013, 15, 125-136.
- (28) Zhu, J.; Cole, R. B. J. Am. Soc. Mass Spectrom. 2000, 11, 932-941.
- (29) Cole, R. B.; Zhu, J. Rapid Commun. Mass Spectrom. 1999, 13, 607—611.
- (30) Bowie, J. H. Mass Spectrom. Rev. 1990, 9, 349-379.
- (31) Sheng, H.; Tang, W.; Gao, J.; Riedeman, J. S.; Li, G.; Jarrell, M. T.; Hurt, R. M.; Yang, L.; Murria, P.; Nash, J. J.; Kenttamaa, I. H. *Anal. Chem.* 2017, 89, 13089–13096.