

# Novel Method for the Determination of the Methoxyl Content in Lignin by Headspace Gas Chromatography

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**ABSTRACT:** The paper reports on a headspace gas chromatographic (HS-GC) method for the determination of methoxyl in lignin. The method involves the quantitative cleavage of methoxyl with hydroiodic acid (HI) to form methyl iodide in a closed headspace sample vial at 130 °C for 30 min. After HI has been added, the sample is neutralized by injecting a sodium hydroxide solution; the methyl iodide in the vial was determined by HS-GC using a flame ionization detector. The results showed that the method has an excellent measurement precision (RSD < 0.69%) and accuracy (RSD < 3.5%) for the quantification of methoxyl content in lignin. The present method is simple and accurate and can be used for the efficient determination of methoxyl content in lignin and related materials.

**KEYWORDS:** methoxyl, lignin, headspace, gas chromatography, hydroiodic acid

## INTRODUCTION

Among the characteristic groups of lignin, methoxyl is one of the major functional groups of interest and has been studied most extensively.<sup>1</sup> The content of methoxyl in lignin is quite different because of the differences of the chemical structures in the variety of lignocellulosic materials. It is well-known that the methoxyls associated with phenylpropane, syringyl and guaiacyl units, have different reactivities in chemical reactions.<sup>2</sup> Therefore, the analysis of the methoxyl content of these lignin samples can provide not only reliable structural information (e.g., the ratio of syringyl to guaiacyl) but also a good indication of the structural changes during a chemical treatment.<sup>3–5</sup> Such information is very important in delignification studies aiming at selectively separating cellulose from lignin in lignocellulosic materials.<sup>6</sup> Therefore, a method for the accurate determination of methoxyl content in lignin plays an important role in both the lignin chemical structure analysis and the research of lignin structural change during pulping and bleaching processes.

Currently, volumetric titration based on iodometry<sup>7,8</sup> is a dominant and practical technique used for the determination of methoxyl content in lignin. In this method, the following two steps are required before the titration: (1) Cleavage of the methoxyl group from lignin via hydroiodic acid (HI) forms methyl iodide, that is



(2) Methyl iodide is distilled out from the above reaction medium and trapped in a bromine–glacial acetic acid–potassium acetate solution, and then formic acid is added to destroy excess bromine. Clearly, these steps and the titration procedure are not only complicated and time-consuming but also subject to large errors. Although advanced techniques such as gas chromatography (GC)<sup>9</sup> have been also used for the quantification of methyl iodide generated from reaction 1, the large uncertainty caused in the methyl iodide sampling is a major problem due to its high volatility. As a result, it significantly affects the accuracy in methyl iodide measurement.

Headspace gas chromatography (HS-GC) has been widely used for the analysis of volatile species in complex matrix samples.<sup>10–15</sup> HS-GC can also be used to indirectly analyze some nonvolatile compounds that could be converted to their corresponding volatile species through chemical reactions.<sup>16–19</sup> Because reaction 1 can be performed in a closed headspace sample vial, the formed methyl iodide after the reaction can be measured by HS-GC without any manual sampling step as mentioned in the GC method.<sup>9</sup> In this way, the large error caused mainly by sample handling can be minimized.

In this work, we developed a novel HS-GC method for determination of the methoxyl content in lignin. The main focuses were to explore the reaction conditions (i.e., the dosage of HI, temperature, time, and sample size) on the completeness of methoxyl conversion and the operation conditions for headspace equilibration in the present method.

## MATERIALS AND METHODS

**Chemicals.** All chemicals used in the experiment were from commercial sources; hydroiodic acid (57%) and methyl iodide were of analytical grade and purchased from Sigma-Aldrich. The lignin samples were obtained from our laboratory.

**Apparatus and Operation.** All measurements were carried out using an HSS 80.65 automatic headspace sampler (DANI, Italy) and model GC-2010 capillary gas chromatograph (Shimadzu, Japan). GC conditions were as follows: DB-5 capillary column (30 m × 0.25 mm × 0.1 μm) at 30 °C, high-purity nitrogen carrier gas flow rate of 3.8 mL/min. A flame ionization detector (FID) was employed with hydrogen and air with flow rates of 40 and 400 mL/min, respectively. The detector temperature was 250 °C. Headspace operating conditions were as follows: 1 min strong shaking to obtain sample at a temperature of 50 °C, vial pressurization time of 0.2 min, sample loop (3 mL) fill time of 1.0 min, loop equilibration time of 0.05 min, and injection time of 0.3 min.

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**Sample Preparation and Measurement Procedures.** Half a milliliter of hydroiodic acid (57%) was mixed with solid sample (10.0–20.0 mg) in a headspace test vial (21.6 mL), which was then sealed with a septum. The vials were placed in an oil bath and heated for various periods of time (range of 10–120 min) at 130 °C. The vials were cooled to room temperature, and 0.5 mL of sodium hydroxide solution (6 mol/L) was injected into the sealed vial by a syringe to neutralize the excess hydroiodic acid. The vial was then immediately put into the headspace autosampler for HS-GC measurement.

## RESULTS AND DISCUSSION

**Effects of Reaction Conditions on the Conversion of Methoxyl Groups.** The reaction conditions for converting methoxyl in lignin to methyl iodide were similar to those used in the volumetric titration method.<sup>8</sup> To avoid any possible loss of methyl iodide formed from the reaction, the process was in the present method performed in a closed headspace sample vial.

For a complete conversion from methoxyl group to methyl iodide, an excess amount of hydroiodic acid is necessary. According to Figure 1, 0.15 mL of hydroiodic acid (57%) is sufficient to achieve a complete reaction for any lignin compounds at the given conditions.

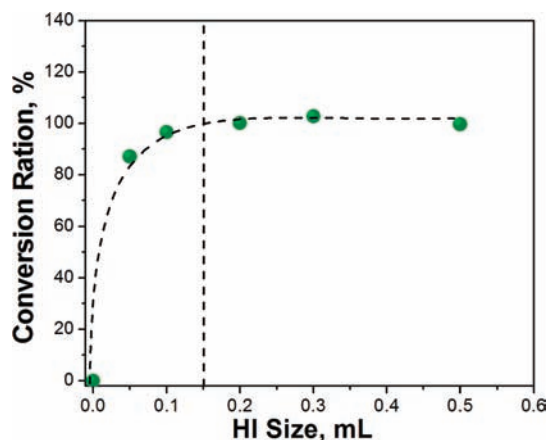


Figure 1. Effect of hydroiodic acid size on methoxyl group conversion.

As shown in Figure 2, a complete conversion can be achieved at 130 °C for 30 min in a closed sample vial. This result indicates that the pressurized condition is helpful for accelerating the reaction, which is much quicker than the

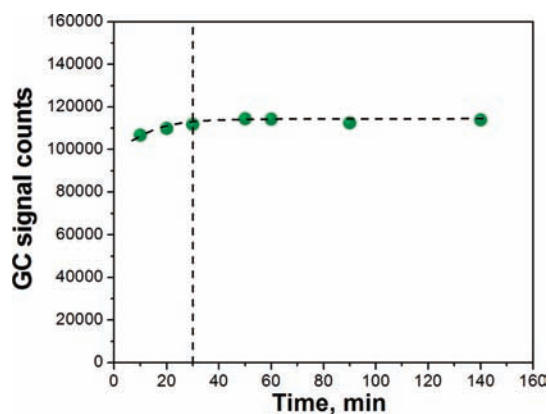


Figure 2. Effect of reaction time on methoxyl group conversion (temperature = 130 °C).

titration method.<sup>8</sup> In the present method, the reaction for converting the methoxyl group in lignin to methyl iodide was performed in the oven, which is also practical and efficient in the massive sample pretreatment (reaction).

**Neutralization of Hydroiodic Acid.** Because reaction 1 is performed in a hydroiodic acid (a volatile acid) medium, the corrosion on headspace sampling channel<sup>20,21</sup> could be a big problem in the application of the method. In the present method, a 0.5 mL of sodium hydroxide solution (6 mol/L) solution was injected into the sealed sample vial by a syringe to neutralize the excess hydroiodic acid after the methoxyl conversion reaction. Thus, the hydroiodic acid was completely converted to sodium iodide form in the resulting solution, and the corrosion problem in the sampling channel of the autoheadspace sampler was eliminated.

**Conditions for Headspace Analysis.** The headspace analysis is based on vapor–liquid equilibrium (VLE) of methyl iodide in a closed sample vial; thus, the total mass of the analyte in the vial can be determined according to its vapor concentration measured by GC. Figure 3 shows that the methyl iodide of the sample from the reaction can be well-separated in the HS-GC measurement.

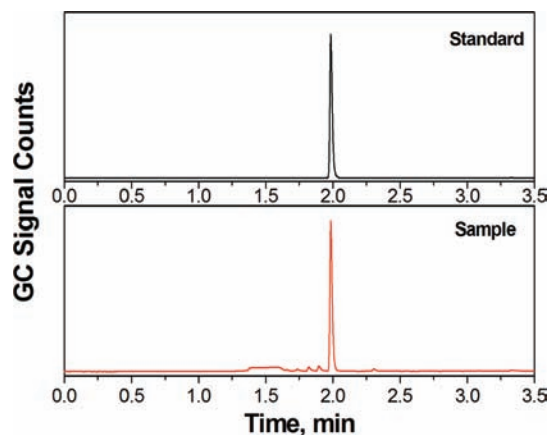


Figure 3. HS-GC chromatogram from methyl iodide in reference standard and a sample analysis.

For an efficient analysis, a short VLE time is desired. As shown in Figure 4, the VLE can be achieved within 2 min at the given temperature (50 °C). Therefore, we chose a time of 2 min for the rest of the study.

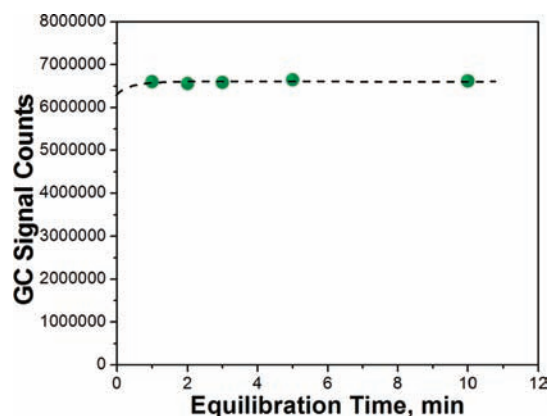


Figure 4. Effect of equilibration time.

The VLE partition coefficient is a function of temperature. The effect of the equilibration temperature is shown in Figure 5. It can be seen that the two-phase equilibrium is achieved by 2 min for a sample at a temperature above 45 °C.

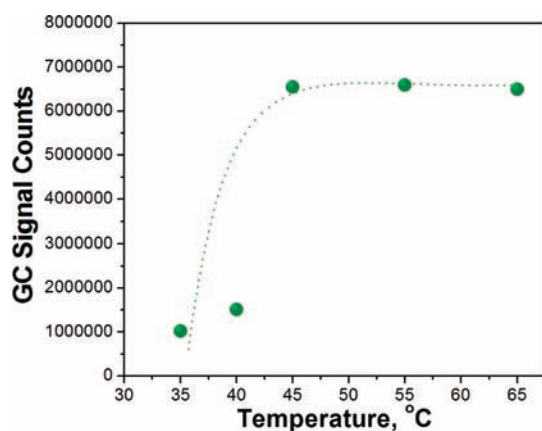


Figure 5. Effect of equilibration temperature.

**Method Calibration, Precision, and Validation.** The repeatability testing of the present method was studied. The results show that the relative standard deviation (RSD) in five measurements is <0.69% (as shown in Table 1), in which the random errors are associated with the uncertainties in both sampling and HS-GC measurement.

Table 1. Repeatability Testing

sample	methoxyl content, %
1	4.98
2	5.04
3	5.03
4	4.96
5	5.02
average	5.01
RSD, %	0.69

A simple external standard calibration can be employed in the HS-GC method. In this work, the calibration was performed on the basis of the addition of different volumes (0–10  $\mu$ L) of pure methyl iodide in a set of headspace sample vials, and then the samples were tested by HS-GC. According to the data from GC measurement on these samples, a standard calibration curve was obtained, that is

$$A = 295.7(\pm 4.5) \times C - 8.1(\pm 49.8) \quad (2)$$

where A and C represent the GC signal count of methyl iodide and its absolute amount (in  $\mu$ g) in the headspace sample vial, respectively. The regression coefficient  $R^2 = 0.999$  ( $n = 6$ ). The limit of quantitation (LOQ) and limit of detection (LOD)<sup>22</sup> in the present method are 1.66  $\mu$ g and 0.53  $\mu$ g, respectively.

To verify the present method, four lignin samples with known methoxyl contents (measured by the titration method) were used. As shown in Table 2, the results of the methoxyl content testing of these samples by the present method match well those known by the titration method (RSD < 3.5%), indicating that the present technique is justifiable for the determination of methoxyl content in lignin samples.

Table 2. Comparison of the Methods

sample	methoxyl content, %		RSD, %
	titration	HS-GC	
L-1	3.17	3.26	2.80
L-2	5.60	5.52	-1.44
L-3	7.81	7.89	1.02
L-4	10.00	10.35	3.38

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### Notes

The authors declare no competing financial interest.

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