**Editorial comments:**  
The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (55165\_R1\_071116.docx) is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink for downloading the .docx file. Please download the .docx file and use this updated version for any future revisions.  
  
1. Grammar:  
-3.2.9 – “th eendpoint”

Response: To address other comments, this sentence has been changed in the manuscript. See response to the following comment for 3.2.9.

-3.2.9, 3.3.6, 3.4.6, 3.4.12.– Please correct the run-on sentence.

Responses:

3.2.9: The sentence has been changed to the following: “Transfer the sample to a titration vessel, washing the reaction vial several times separately with ethanol and water in proportions to make a final 80% solution of ethanol/water. Titrate with the acid solution using an automatic titrator to the endpoint. Record the endpoint.”

3.3.6: The sentence has been changed to the following: “Transfer the sample to a titration vessel, washing the reaction vial several times separately with ethanol and water in proportions to make a final 80% solution of ethanol/water. Titrate with the acid solution using an automatic titrator to the endpoint. Record the endpoint.”

3.4.6: The sentence has been changed to the following: “Transfer the sample to a titration vessel, washing the reaction vial several times separately with ethanol and water in proportions to make a final 80% solution of ethanol/water. Titrate with the acid solution using an automatic titrator to the endpoint. Record the endpoint.”

3.4.12: The sentence has been changed to the following: “Transfer the sample to a titration vessel, washing the reaction vial several times separately with ethanol and water in proportions to make a final 80% solution of ethanol/water. Titrate with the acid solution using an automatic titrator to the endpoint. Record the endpoint.”

This change was also made for 3.1.3. Section 3.1.3 has been changed to the following: “Transfer the sample to a titration vessel, washing the reaction vial several times separately with ethanol and water in proportions to make a final 80% solution of ethanol/water. Titrate with the acid solution using an automatic titrator to the endpoint. Record the endpoint.”

-4.1.1 – Please correct the sentence structure, using imperative tense.

Response:

4.1.1: The sentence has been changed to the following: “Calculate the concentration of the acid solution (mol/L) using the following equation. The weight of the dry sodium carbonate in grams is w1, the purity is written as a fraction (i.e., 99% is 0.99), and the endpoint is in mL.”

4.2.1: The sentence has been changed to the following: “Calculate the concentration of 4BBA (mol/L) in the sample using the following equation. The weight of the 4BBA in grams is w2, the 4BBA purity is written as a fraction (i.e., 99% is 0.99), the concentration of the acid solution is [acid], the triethanolamine/hydroxylamine•HCl blank endpoint is EPBA (the average value of three blanks, in mL) and the endpoint is EP (in mL).”

4.3.1: The sentence has been changed to the following: “Calculate the concentration of carbonyls in bio-oils [CO] (mmol/g-bio oil) using the following equation. The weight of the bio-oil in grams is w3, the concentration of the acid solution (mol/L) is [acid], the triethanolamine/hydroxylamine•HCl blank endpoint is EPBA (the average value of three blanks in mL), and the endpoint is EP (in mL).”

-Line 2.4.4 – “addition of alcohols cause”

Response: This has been changed to “addition of alcohols causes”

2. Additional detail is required:  
-2.3 – Is a specific container used to achieve this?

Response: No. This is a general comment for the handling and storage of bio-oils, and is common practice.

-3.1.3 – Please describe how to “quantitatively transfer.”

Response: The statement “quantitatively transfer” has been changed to “Transfer the sample to a titration vessel, washing the reaction vial several times separately with ethanol and water in proportions to make a final 80% solution of ethanol/water.” As outlined above, this was changed for 3.1.3, 3.2.9, 3.3.6, 3.4.6, and 3.4.12.  
  
3. Results:  
-Please include a brief description in the figure legend of Figure 1.

Response: The following description was added: “Reaction schematic showing the conversion of a carbonyl compound to the corresponding oxime.”

-Please include results from a representative experiment showing that the carbonyl compounds have been quantified and the values that were obtained. These results should be discussed in the results section. This data should be supplied as a graph with appropriate error bars.

Response: Figure 2 has been revised to include the endpoint values for both blank titrations and pyrolysis oil titrations. For clarity, we have decided to only show a single representative titration curve for each, as triplicate titration curves would make the figure too confusing due to the variation in sample weight and endpoint location of the triplicates. The revised Figure 2 has a table inset showing triplicate data for both blank and pyrolysis oil titrations, with average and standard deviation values reported.

Additionally, the following text has been added in the Discussion section: “Representative titration curves are shown in Figure 2. A blank titration, as well as a titration for a pyrolysis oil sample, are shown. Furthermore, the first derivative of the titration curve (dpH/dV) is shown, which allows for easy recognition of the titration endpoint. The inset table on Figure 2 shows triplicate data for both pyrolysis oil and blank titrations, with average values and standard deviations. The endpoint values shown (in mL) are used in Section 4 to calculate the total carbonyl content (in mmol/g) in the pyrolysis oil sample. For the pyrolysis oil titration shown in Figure 2, the bio-oil sample mass was 0.1148 g, the acid concentration was 0.07032 mol/L, the blank endpoint was 13.041 mL, and the pyrolysis oil endpoint was 4.891 mL. This resulted in a calculated carbonyl content of 4.992 mmol/g bio-oil.”

4. Discussion: Please discuss the limitations and future applications of the protocol.

Response: In addition to the limitations already discussed in the Discussion section, the following text has been added on line 276: “This method has been used to reliably analyze at least 20 raw pyrolysis bio-oils. Additionally, several hydrotreated pyrolysis bio-oils have been successfully analyzed. The applicability of this method to a wide variety of bio-oil samples, combined with a high level of accuracy and reliability, may lend this method to other applications in the future. For example, carbonyl content may be used to replace viscosity in a bio-oil aging test.”  
  
**Reviewers' comments:**  
**Reviewer #1:**  
*Manuscript Summary:*  
This is well written manuscript and is recommended for acceptance.  
  
*Major Concerns:*  
N/A  
  
*Minor Concerns:*  
N/A  
  
*Additional Comments to Authors:*  
N/A  
  
  
**Reviewer #2:**  
*Manuscript Summary:*  
This method measure the quantity of carbonyls in the thermolysis-prepared bio-oils.  
  
*Major Concerns:*  
This method is quite useful to measure the chemical functionalities of bio-oil.  
1. Line 100-103: Homogenizing bio-oil is not easy for a lot of "dirty" bio-oils. Shaking just for several minutes may not be enough. The use of this method may be limited for the "cleaner" bio-oils without many dirty oslid particles and tars. In many cases, the raw bio-oils exhibit the multi-phasic (more than biphasic) complex mixture. This method may be more useful for the hydrotreated or upgraded bio-oils.

Response: We have found this method applies to a large variety of pyrolysis bio-oils. We have tested on the order of 20 different raw pyrolysis bio-oil samples, as well as several hydrotreated samples, and found that the method worked well for all bio-oil samples tested.

The following text has been added to the Discussion: “This method has been used to reliably analyze at least 20 raw pyrolysis bio-oils, both fresh and aged up to 120h at 80°C. Additionally, several hydrotreated pyrolysis bio-oils have been successfully analyzed. The applicability of this method to a wide variety of bio-oil samples, combined with a high level of accuracy and reliability, may lend this method to other applications in the future. For example, carbonyl content may be used to replace viscosity in a bio-oil aging test.”

The homogenization instructions have been changed in 2.1 to the following: “Make sure the oil sample is at room temperature prior to withdrawing a sample. Thoroughly homogenize (mix by shaking vigorously for at least 1 minute, and visually inspect the sample to ensure it is homogenous. Some bio-oils may require longer shaking times) bio-oil to obtain a representative sample.”

2. Regarding above question, the authors did not mention the source of their bio-oil. Preparation methods and biomass resources must be mentioned.

Response: Details of the bio-oil used in method development are in Reference 9, but we have tested this method on a large variety of pyrolysis bio-oils, both raw and upgraded. For this JoVE manuscript, we were not aware we had to list the specifics of bio-oils tested, and as this method applies to different types of pyrolysis bio-oils, we were hoping to keep the method more general by not listing specifics of bio-oil production. Our explanation of the applicability of this method to different types of bio-oils was described in lines 74-78 in the Introduction.  
  
*Minor Concerns:*  
1. For the more accurated description, the names of reagents in the manuscript should be arranged to be the same "formal" names. The authors used "ethyl alcohol" and "ethanol" at the same time.

Response: “Ethyl alcohol” at the beginning of the protocol section (Line 82) has been changed to “ethanol”.  
  
*Additional Comments to Authors:*  
N/A

Other changes made: Line 322 – reference 10 was updated as volume and page numbers are now available for this reference.