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## Laboratory Analytical Procedure (LAP)

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**Technical Report**  
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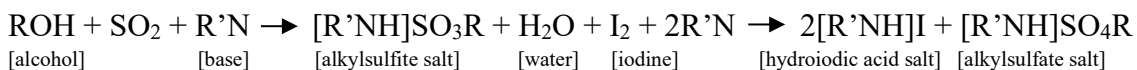
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## 1. Introduction

- 1.1 The water content of bio-oils is a key metric for several reasons. Water is typically the most concentrated single component of fast pyrolysis oils, though this will be much lower in catalytic fast pyrolysis and upgraded products. Reduced water content is preferable in bio-oil as water can contribute to phase separation, corrosivity, and instability. Additionally, lower water is beneficial for physical properties such as energy density [1,2]. The percentage of water can also be used to correct the calculation for organic oxygen when determining total oxygen content of bio-oils via combustion-based ultimate analysis.
- 1.2 The procedure described here is specifically written for the analysis of bio-oils, and is based on ASTM E203, Standard Test Method for Water Using Volumetric Karl Fischer Titration [3]. This standard test method is specified for measuring the mass % water in pyrolysis liquid biofuels in ASTM D7544, Standard Specification for Pyrolysis Liquid Biofuel [4]. Although the standard method is prescribed for the analysis of pyrolysis liquids for use in industrial and commercial burners, the method is not specifically written for this product. The method described here provides specific guidance for the analysis of bio-oils. Both organic and aqueous phases can be measured with this technique.
- 1.3 This procedure uses a *one-component* titrant (all reactants are in one solution). The recommended titrant is CombiTitrant 5 which contains base (imidazole), iodine (I<sub>2</sub>), and SO<sub>2</sub> and has a standard strength of 5 mg H<sub>2</sub>O/mL. Any one-component titrant of equivalent quality is acceptable for use. The titration medium (alcohol) used is methanol. Titration medium is conditioned with titrant to remove small amounts of pre-existing water prior to addition of sample. Sample is then added gravimetrically using a volumetric pipette and the resistance of the cell is monitored continuously with a platinum electrode to determine the titration end point. The general reaction is provided below in Scheme 1.

Scheme 1:



## 2. Scope

- 2.1 The method described here was developed to analyze the concentration of water in fast pyrolysis oil (FP), catalytic fast pyrolysis oil (CFP), and aqueous phases isolated from these products. Other products can be measured using this technique provided that water concentration is within the approximate range of 0.1 to 100 wt%. Lower concentrations of water can be measured using coulometric Karl Fischer methods.

### 3. Terminology

- 3.1 *Titer Water Calibration Standard*: A standard with a known amount of water. Used to determine the titer of the CombiTitrant 5.
- 3.2 *Bio-oil*: The crude liquid product of converting solid biomass into a liquid via fast pyrolysis or other thermochemical conversion process.
- 3.3 *Pyrolysis* – Chemical decomposition of organic materials by heating in the absence of oxygen.
- 3.4 *Fast Pyrolysis (FP)* – Pyrolysis conducted with rapid heating and short residence time; typically, less than 10 seconds.
- 3.5 *Catalytic Fast Pyrolysis (CFP)* – Fast pyrolysis conducted in the presence of a catalyst designed to perform partial deoxygenation.

### 4. Interferences

- 4.1 The CombiTitrant 5 recommended in this method can change concentration over time due to absorption of atmospheric water. The concentration must be determined experimentally prior to analysis and must be very close to the reported concentration from the manufacturer or the percentage water measurement will not be accurate.
- 4.2 Methanol used as the titration medium can absorb water from the atmosphere during solvent transfer to the titration cell. Most Karl Fischer instruments have desiccant filled drying tubes connected to the solvent, titrant, and titration vessel to prevent moisture entering any of the reagents. Desiccant must be replaced regularly to limit interference from atmospheric moisture.
- 4.3 High volatility samples may evaporate during introduction if this is not done quickly. All sample weighing and introduction into the titration vessel must be done as quickly as possible. Care must be taken to ensure all sample is introduced into the titration vessel to provide an accurate sample weight.
- 4.4 It is important to have all samples as homogenous as possible to reflect the true percentage of water in the sample. Shake samples at ambient temperature vigorously before weighing and introduction into the titration vessel to ensure a representative sample.
- 4.5 The titer water calibration standard used to check the titrant should not be left open as solvent evaporation and water absorption from the atmosphere can change the water concentration and lead to erroneous results.

## 5. Apparatus

- 5.1 Analytical balance, accurate to 0.1 mg or better
- 5.2 Karl Fischer Instrument (Metrohm 870 KF Titrino plus or equivalent)

## 6. Reagents and Materials

### 6.1 Reagents

- 6.1.1 Methanol (anhydrous, suitable for Karl Fischer Reagent, 0.1% water max.) (CAS # 67-56-1)
- 6.1.2 CombiTitrant 5 (5 mg/mL, Sigma Aldrich Cat # 1880051000 or comparable)
- 6.1.3 Water standard: HYDRANAL – Water Standard 10.0, 10 mg/g (1.0%) (Honeywell Research Chemicals Cat # 34849, or comparable)

### 6.2 Materials

- 6.2.1 Microdispenser Pipette (1000  $\mu$ L) (VWR Cat # 53506-223, or comparable)
- 6.2.2 Microdispenser Pipette (100  $\mu$ L) (VWR Cat # 53506-201, or comparable)
- 6.2.3 Capillary Bores (1000  $\mu$ L, for microdispenser) (VWR Cat # 53508-660, or comparable)
- 6.2.4 Capillary Bores (100  $\mu$ L, for microdispenser) (VWR Cat # 53508-499, or comparable)
- 6.2.5 Small (20 mL) screw cap headspace vial with PTFE lined cap to temporarily hold titer water calibration standard

## 7. Environmental Safety and Health Considerations and Hazards

- 7.1 Methanol is flammable and toxic.
- 7.2 CombiTitrant (skin irritation, serious eye damage, suspected of causing cancer, causes damage to organs through prolonged exposure).
- 7.4 HYDRANAL – Water Standard 10.0 (skin irritation, serious eye damage, causes damage to organs through prolonged exposure, flammable).
- 7.5 Pyrolysis bio-oils and solvents should be handled in a fume hood.

- 7.6 Follow all applicable chemical handling procedures.
- 7.7 Follow all applicable waste disposal and handling procedures.

## 8. Sampling, Test Specimens, and Test Units

- 8.1 Allow samples to reach room temperature before analyzing.
- 8.2 Samples should be thoroughly homogenized prior to sampling for titration to obtain a representative sample.
- 8.3 Limit air exposure of samples to prevent degradation and absorption of atmospheric water.

## 9. Analytical Procedure

- 9.1 Performing Titer to confirm concentration of CombiTitrant 5.

**Note:** The following procedure was written for a Metrohm 870 KF Titrino plus Karl Fischer Instrument. Adjust the procedure accordingly for other instruments. Contact the instrument manufacturer for specific operation steps. The basic procedure of conditioning the medium, analyzing the calibration standard, and analyzing samples will follow the same steps regardless of instrument manufacturer.

- 9.1.1 Turn on Karl Fischer Instrument, fill titration vessel with methanol until electrodes are completely covered and select titer method. For 870 Titrino this is “Titer I<sub>pol</sub>”.
- 9.1.2 Condition titration medium (water removed from medium by titrating with CombiTitrant 5). With most modern Karl Fischer Instruments, the methanol medium in the titration vessel should automatically be conditioned after the method has been selected (“Titer I<sub>pol</sub>”). Determine how to condition your titration vessel if it does not condition automatically.
- 9.1.3 Transfer titer water calibration standard to a suitable container for use (20 mL screw cap headspace vial recommended).  
Note: calibration standards are typically provided in sealed ampules to prevent evaporation and water absorption before use. Use care when breaking the ampule.
- 9.1.4 After conditioning the titrant medium perform the following steps to analyze the titer water calibration standard.
  - 9.1.4.1 Adjust 1000 µL microdispenser pipette to 800 µL.
  - 9.1.4.2 Fill microdispenser with standard and remove excess on outside of pipette tip when pulling it out of the solution. This prevents weight



change due to standard dripping off or evaporating while weighing standard.

- 9.1.4.3 Put the microdispenser on the scale, with plunger side down so it can balance with pipet tip upright. A beaker or similar container can also be used to hold microdispenser upright while it is on the scale.
  - 9.1.4.4 Press start on instrument (It will have a countdown instructing you to add sample).
  - 9.1.4.5 Immediately tare scale and ensure it remains stable at 0.
  - 9.1.4.6 Immediately add sample to titration medium. Make certain the sample is injected into the titration medium and no sample is lost to the walls of the titration vessel. Do not touch the pipette tip to the surface of the titration medium, as this can cause transfer of medium to pipette and error in sample weight.
  - 9.1.4.7 Place the microdispenser back on scale to obtain sample weight.
  - 9.1.4.8 Press “Enter” on KF instrument keypad and enter the weight from the scale. (Note this will be a negative number as this is determining the mass lost by injection).
  - 9.1.4.9 Press “Enter” on keypad when done entering the sample weight and then press “Start” on instrument and let titration proceed.
- 9.1.5 Perform the above water standard procedure in triplicate. The standard value of the CombiTitrant is 5 mg H<sub>2</sub>O/mL. For accurate water measurement it is recommended that the average value should be no less than 4.8 mg H<sub>2</sub>O/mL, as lower values may indicate the titrant has degraded or become contaminated. The percent relative standard deviation (RSD) of the triplicate analyses shall be ≤ 0.5%. Variability greater than this indicates potential problems with sample weighing/introduction or the instrument dispensing titrant. If the RSD is higher than 0.5% this analysis shall be repeated. If the value is again greater than 0.5% the cause shall be corrected to eliminate sources of imprecision prior to analysis of samples.

## 9.2 Water mass % determination of organic phase (bio-oil).

- 9.2.1 Select KF measurement method on instrument (“KF Ipol” for Titrino 870).
- 9.2.2 Set the 100 µL microdispenser pipette to 100 µL and obtain 100 µL of bio-oil sample. Use a Kim Wipe or similar to wipe off excess sample on outside of pipette tip, taking special care near the tip opening to not accidentally absorb any material that is inside the pipette tip.

- 9.2.3 Follow the same procedure as in 9.1.4.3 through 9.1.4.9.
- 9.2.4 Perform the procedure in triplicate and determine the average value, standard deviation, and % RSD of the triplicate determinations.

### 9.3 Water % determination of aqueous phase.

- 9.3.1 Select KF measurement method on instrument (“KF Ipol” for Titrino 870).
- 9.3.2 Set the 100  $\mu\text{L}$  microdispenser pipette to 30  $\mu\text{L}$  and obtain 30  $\mu\text{L}$  of aqueous sample. Use a Kim Wipe or similar to wipe off excess sample on outside of pipette tip, taking special care near the tip opening to not accidentally absorb any material that is inside the pipette tip.
- 9.3.3 Follow the same procedure as in 9.1.4.3 through 9.1.4.9.
- 9.3.4 Perform the procedure in triplicate and determine the average value, standard deviation, and % RSD of the triplicate determinations.

## 10. Report Format

- 10.1 Report results to the nearest decimal point. Provide the mean value, standard deviation, and % RSD of the titer determination, organic phase (bio-oil), and aqueous phase (if applicable).

## 11. Precision and Bias

- 11.1 The interlaboratory precision of this method has not yet been determined. The precision of this method will be updated once an interlaboratory study has been conducted.
- 11.2 Replicate analyses of FP, CFP, and aqueous phase of CFP were conducted to provide interim intra-laboratory precision. Thirty independent analyses were conducted on each sample over the course of three days. The resultant 95% confidence interval is as follows:

$$r = (X \times 0.022) + 0.067$$

Where:

r = repeatability, the difference between replicate analyses

X = the average mass % water of two replicate analyses

The difference between two analyses would be expected to exceed r only for one case in 20.

## 12. Quality Control

- 12.1 Fresh titration medium shall be used at the start of each analytical batch.
- 12.2 To ensure proper operation the titer water calibration standard should be analyzed at the start of each day of operation and checked periodically to confirm accuracy.
- 12.3 It is recommended that a representative sample of known composition be analyzed with each sample batch to confirm accuracy.
- 12.4 Replicates: Run all samples in triplicate.

## 13. References

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- [2] Westerhof, Roel J.M., et al., *Controlling the Water Content of Biomass Fast Pyrolysis Oil*. Eng. Chem, 2007. 46: 9238-9247.
- [3] ASTM E203-16, *Standard Test Method for Water Using Volumetric Karl Fischer Titration*. ASTM International: West Conshohocken, PA.
- [4] ASTM D7544-12R17, *Standard Specification for Pyrolysis Liquid Biofuel*. ASTM International: West Conshohocken, PA.