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Laboratory Analytical Procedure (LAP)

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March 2016

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1. Introduction

1.1 This laboratory analytical procedure covers the quantification of semi-volatile oxygenated compounds present in fast pyrolysis bio-oils. During an inter-laboratory validation study, this method showed variabilities on the order of 20% RSD for some compounds [2]. These variabilities must be considered when using this method to compare bio-oils produced at different facilities.

1.2 Oxygenated compounds present in bio-oils are derived from both polysaccharides and lignin. These compounds include carboxylic acids, phenolics, carbonyls, and multifunctional oxygenates. Knowledge of these compounds and their concentrations can assist in determining impacts on bio-oil composition from changing feedstock composition or process variables. Furthermore, quantification of individual compounds in bio-oil can be useful for downstream processing such as upgrading processes.

2. Scope

2.1 This procedure is developed specifically for analysis of fast pyrolysis bio-oil derived from lignocellulosic feedstocks. Hydrotreated products and bio-oils derived from other processes and feedstocks may not contain significant quantities of the compounds included in this method.

2.2 This methodology covers many compounds commonly detected in relatively high concentration in pyrolysis bio-oils. These compounds have been included due to their known presence in bio-oils and availability for purchase in high purity. Many additional compounds are known to be present in bio-oils and this method does not provide quantification of all compounds detectable by GC-MS.

3. Terminology

3.1 *Bio-oil* – The crude liquid product of converting solid biomass into a liquid via fast pyrolysis or other thermochemical conversion process. For the purposes of this methodology bio-oil will refer specifically to products of fast pyrolysis.

3.2 *Internal Standard* – A known amount of a compound known to be absent from pyrolysis oils is added to all calibration standards and samples. The instrument response of this standard is used to correct for any instrument variability or solvent evaporation during analysis.

4. Interferences

4.1 Bio-oils are known to degrade over time when exposed to oxygen and heat. Samples that have aged may not be representative of the original bio-oil product.

5. Apparatus

5.1 Analytical balance accurate to 0.1 mg

5.2 Ultrasonic bath (Branson 3510 or equivalent)

5.3 Gas Chromatograph (GC) equipped with split/splitless injection port (Agilent 7890A or equivalent)

5.4 Automatic liquid sampler compatible with GC system (Agilent 7693A or equivalent)

5.5 Mass Selective Detector (MSD or MS) with electron ionization (EI) source and linear quadrupole mass analyzer (Agilent 5975C or equivalent)

5.6 Capillary column with 14%-cyanopropyl-phenyl-methylpolysiloxane stationary phase: Restek Rtx-1701 of dimensions 60 m length x 250 µm internal diameter, 0.25 µm film thickness, or equivalent

5.7 Split injection port liner. Single taper, low pressure drop, deactivated with glass wool (Agilent 5183-4647 or equivalent)

6. Reagents and Materials Needed

6.1 Reagents

6.1.1 Acetonitrile, high-performance liquid chromatography (HPLC) grade (Sigma-Aldrich 34998 or equivalent)

6.1.2 Isoamyl ether (Sigma-Aldrich 260649 or equivalent)

6.1.3 1-Octanol (Sigma-Aldrich 293245 or equivalent)

6.1.4 Methyl Laurate (Sigma-Aldrich 234591or equivalent)

6.1.5 Glycolaldehyde(Sigma-Aldrich G6805 or equivalent)

6.1.6 Glacial acetic acid (Sigma-Aldrich 320099 or equivalent)

6.1.7 Acetol (Alfa Aesar L15008 or equivalent)

6.1.8 3-Hydroxy-2-butanone (Sigma-Aldrich 40127-U or equivalent)

6.1.9 Propanoic acid (Sigma-Aldrich 402907 or equivalent)

6.1.10 Butanoic acid (Sigma-Aldrich 19215 or equivalent)

6.1.11 2-Cyclopenten-1-one (Sigma-Aldrich C112909 or equivalent)

6.1.12 Furfural (Sigma-Aldrich 185914 or equivalent)

6.1.13 5-Methylfurfural (Sigma-Aldrich 137316 or equivalent)

6.1.14 2(5H)-Furanone Sigma-Aldrich 283754 or equivalent

6.1.15 3-Methyl-1,2-cyclopentanedione, (Sigma-Aldrich 178500 or equivalent)

6.1.16 3-Methyl-2(5H)-furanone (TCI M1078 or equivalent)

6.1.17 Phenol (Sigma-Aldrich P5566 or equivalent)

6.1.18 Guaiacol (TCI M0121 or equivalent)

6.1.19 o-Cresol (Sigma-Aldrich C85700 or equivalent)

6.1.20 Maltol (Sigma-Aldrich W265608 or equivalent)

6.1.21 p-Cresol (Sigma-Aldrich C85751 or equivalent)

6.1.22 m-Cresol (Sigma-Aldrich C85727 or equivalent)

6.1.23 Creosol (Sigma-Aldrich 302880 or equivalent)

6.1.24 2,4-Xylenol (Sigma-Aldrich D174203 or equivalent)

6.1.25 4-Ethylguaiacol (Sigma-Aldrich W243604 or equivalent)

6.1.26 Eugenol (Sigma-Aldrich E51791 or equivalent)

6.1.27 5-Hydroxymethylfurfural (Sigma-Aldrich W501808 or equivalent)

6.1.28 Catechol (Sigma-Aldrich 135011 or equivalent)

6.1.29 Syringol (Sigma-Aldrich D135550 or equivalent)

6.1.30 Vanillin (Sigma-Aldrich V1104 or equivalent)

6.1.31 Hydroquinone (Sigma-Aldrich H3660 or equivalent)

6.1.32 Apocynin (Sigma-Aldrich A10809 or equivalent)

6.1.33 Levoglucosan (Sigma-Aldrich 316555 or equivalent)

6.1.34 Syringylaldehyde (Sigma-Aldrich S7602 or equivalent)

6.1.35 Acetosyringone (Sigma-Aldrich D134406 or equivalent)

6.2 Materials

6.2.1 Gas-tight syringes covering ranges from 5 – 1,000 μL

6.2.2 Volumetric flasks (class A), 25 mL

6.2.3 8 mL glass vials with screw caps (Aldrich Z188719 or equivalent)

6.2.4 Glass jars, 30 mL (VWR 82023-838 or equivalent)

6.2.5 1.5 mL GC autosampler vials (Agilent 5182-0543 or equivalent)

6.2.6 Volumetric pipets (class A), 5 mL

6.2.7 PTFE 0.45 µm syringe filters (VWR 28145-493 or equivalent)

6.2.8 Disposable 5mL syringes (VWR 66064-772 or equivalent)

7. ES&H Considerations and Hazards

7.1 Acetonitrile is extremely flammable.

7.2 Acetic, propanoic and butanoic acids are corrosive and flammable.

7.3 Phenol and similar compounds pose significant health hazards.

7.4 Follow all applicable chemical handling procedures.

8. Sampling, Test Specimens and Test Units

8.1 Bio-oil should be allowed to reach room temperature and thoroughly homogenized to obtain a representative sample.

8.2 Exposure to oxygen and heat should be minimized to prevent sample degradation prior to analysis.

9. Analytical Procedure

9.1 Preparation of internal standard solution

9.1.1 Into a 25 mL volumetric flask weigh approximately 30 mg each of isoamyl ether, 1-octanol, and methyl laurate. Record weights to 0.1 mg.

9.1.2 Fill the volumetric flask to the graduation mark with acetonitrile. Invert flask a minimum of three times to mix and pour solution into a labeled 30 mL jar. The solution should be stored in a freezer when not in use.

9.2 Preparation of stock mixed standard

9.2.1 Into a tared 25 mL volumetric flask weigh each of the compounds of interest. Return stopper to flask after adding each compound to reduce evaporative losses. Weigh in order of increasing volatility with the most volatile compounds weighed last. For guidance on standard

preparation refer to ASTM D4037 Standard Practice for Preparation of Liquid Blends for Use as Analytical Standards [1].

9.2.2 The weight of each compound will depend on the anticipated concentration in bio-oil. Acetic acid, glycolaldehyde, and levoglucosan should be weighed at approximately 300 – 400 mg while all other compounds should be weighed at 20 – 30 mg. Amounts can be adjusted to better represent the bio-oil being analyzed.

9.2.3 Fill the volumetric flask to the graduation mark with acetonitrile and close with stopper. Place in ultrasonic bath at 25 °C and sonicate for 5 minutes or until all compounds are fully dissolved. Invert a minimum of three times to mix and transfer solution to a labeled 30 mL jar. The solution should be stored in a freezer when not in use.

9.2.4 Alternatively, a prepared mixed standard can be purchased from a vendor (e.g., Supelco).

9.3 Preparation of calibration standards

9.3.1 Using gas-tight syringes add the following volumes of the stock mixed standard, internal standard solution, and acetonitrile into pre-labeled GC autosampler vials. Cap immediately after adding solutions and solvent to prevent evaporation. Note that volumes can be increased if desired given that ratios of each component are kept constant.

Curve Point	1	2	3	4	5	6	7	8
Stock Standard, µL	25	50	75	100	250	500	750	1000
Internal Standard, µL	100	100	100	100	100	100	100	100
Acetonitrile, µL	975	950	925	900	750	500	250	0

9.3.2 After adding each solution and capping vials, shake each vial to mix.

9.4 Preparation of samples

9.4.1 Weigh approximately 500 mg of sample into a labeled 8 mL vial. Using a gas-tight syringe add 500 µL of internal standard solution. Add 5 mL acetonitrile using a Class A volumetric pipet. Shake thoroughly to dissolve bio-oil.

9.4.2 In order to monitor repeatability of sample preparation a minimum of one sample per sample set, or per 10 samples if a larger number of samples is being analyzed, shall be prepared in triplicate as in 9.4.1. The sample to be tested in triplicate shall be chosen at random from the sample set.

9.4.3 Place diluted samples into ultrasonic bath. Sonicate at 30 °C for 20 minutes to dissolve. Note that the sample may contain a precipitate due to insoluble polymeric material.

9.4.4 Transfer diluted sample to a labeled 1.5 mL GC autosampler vial and seal. If a precipitate is present, filter the sample with a 0.45 µm syringe filter using a 5 mL disposable syringe and transfer filtered sample into autosampler vial.

9.5 GC/MS operation

9.5.1 The GC/MS instrument parameters are as follows:

- Carrier gas: Helium
- Carrier gas flow rate: 1 mL/min (constant flow)
- Injection volume: 1 µL
- Injection port temperature: 250 °C
- Split injection ratio: 30:1
- Initial oven temperature: 45 °C, 10 min hold time
- Oven ramp rate: 3 °C/min
- Final oven temperature: 250 °C, 5 min hold time
- MSD transfer line temperature: 280 °C
- Source temperature: 230 °C
- Quad temperature: 150 °C
- MSD scan range: m/z 29-600
- EI ion source: 70 eV

9.5.2 An acetonitrile solvent blank is analyzed prior to injecting standards or samples to verify the system is free of erroneous peaks, and after each 10^{th} sample injection to monitor for carry over.

9.5.3 The calibration standards are injected in order of lowest to highest concentration. An acetonitrile blank is run after the highest calibration standard to prevent carryover into any samples.

9.5.4 Approximate retention times and appropriate ions used for quantification are listed below for each compound. The internal standards applied to each compound are inserted above each grouping.

Compound	Retention Time (Min)	Quantification ion	Qualifier ion 1	Qualifier ion 2
Isoamyl ether (Internal Standard)	*24.49*	*43*	*70*	*101*
Glycolaldehyde	7.43	31	60	42
Acetic acid	9.06	60	43	45
Acetol	11.18	43	74	103
3-Hydroxy-2-butanone	13.58	45	43	88
Propanoic acid	15.45	74	73	45
Butanoic acid	21.32	60	73	41
2-Cyclopenten-1-one	21.44	82	39	54
Furfural	21.64	96	95	39
5-Methylfurfural	29.86	110	109	53
2(5H)-Furanone	31.50	55	84	54
Octanol (Internal Standard)	*33.41*	*56*	*70*	*84*
3-Methyl-1,2-cyclopentanedione	33.61	112	55	69
3-Methyl-2(5H)-furanone	33.91	41	69	98
Phenol	35.60	94	66	39
Guaiacol	36.20	109	124	81
o-Cresol	37.97	108	107	79
Maltol	38.27	126	71	43
p-cresol	39.69	108	107	79
m-Cresol	39.79	108	107	79
Creosol	38.51	138	123	95
2,4-Xylenol	41.88	122	121	107
Eugenol	48.66	164	149	77
5-Hydroxymethylfurfural	49.42	97	126	41
Catechol	49.75	110	64	92
Syringol	49.92	154	139	111
Methyl laurate (Internal Standard)	*51.82*	*74*	*87*	*55*
Vanillin	54.06	151	152	81
Hydroquinone	54.46	110	81	55
4-Ethylcatechol	56.46	123	138	51
Apocynin	57.29	151	166	123
Levoglucosan	63.28	60	73	57
Syringylaldehyde	64.80	182	181	111
Acetosyringone	67.20	181	196	153

9.5.5 Determine the calibration function and the linearity of the calibration curve for each compound using either the instrument software or by plotting in excel. It is acceptable to use the FORECAST function if plotting in excel. It is highly recommended that the area associated with the quant ion be used for calibration and quantification rather

than TIC. Plot the area ratio and concentration ratio using the area and concentration of the appropriate internal standard. The R^2 of each compound must be 0.995 or greater to be used for quantification. Note that at the concentrations suggested in 9.2.2 the first two curve points may not contain detectable levels of propanoic and butanoic acids. It is acceptable to use the remaining 6 curve points for these two compounds. Catechol may not be detectable in the first three levels and therefore the remaining 5 curve points may be used for this compound.

9.5.6 After injection of the calibration standards the mid-point calibration standard 5 is injected in triplicate to confirm accurate calculation of compound concentrations and injection repeatability. The amount measured should not deviate from actual by greater than 15 %. The percent relative standard deviation (%RSD) should not exceed 2 % for any compound.

9.5.7 Inject samples using the same GC/MS parameters used for standards. Compounds should be identified using their quant ions and qualifier ions to verify correct compounds assignments.

9.5.8 If a sample is found to be too concentrated or too dilute for the calibration curve range, it should be re-prepared at higher or lower weight.

10. Calculations

10.1 Concentration of each component of the internal standard solution and the mixed stock standard:

$$C_1\left(\frac{\mu g}{mL}\right) = \frac{mass\ (mg)\ *\ 1000\ *\ (\frac{\%\ purity}{100})}{25\ mL}$$

10.2 Dilution factor of each calibration curve standard:

$$DF_i = \frac{volume\ of\ standard\ added}{total\ volume}$$

10.3 Concentration of each compound in each calibration curve standard:

$$C_2\left(\frac{\mu g}{mL}\right) = C_1 * DF_i$$

10.4 Sample dilution factor:

$$DF_s = \frac{sample\ weight\ (g) + 3.93\ (g)}{sample\ weight\ (g)}$$

Where:
3.93 g = the mass of 5 mL of acetonitrile

10.5 Internal standard concentration in the sample:

$$C_{IS} = C_1 * DF_s$$

10.6 Using the calibration function determined in 9.5.5 for each component quantify the concentration of individual compounds detected in samples:

$$C_s = [(A_i/A_{IS}) - b]/m * C_{IS} * DF_s$$

Where:
A_i = the peak area of the quantification ion of the individual component
A_{IS} = the area of the quantification ion of the internal standard
b = the intercept of the calibration function
m = the slope of the calibration function

10.7 % Relative standard deviation

$$\%RSD = \left(\frac{\sigma}{mean}\right) * 100$$

Where:
σ = the standard deviation of concentration from replicate analyses
mean = the average concentration determined from replicate analyses

11. Report Format

11.1 Report compound concentrations in µg/mL oil or µg/g of oil.

11.2 Compounds detected at below 80% of the lowest curve point standard concentration should be reported as below limit of quantification.

12. Precision and Bias

12.1 In 2015, an inter-laboratory study was performed on a raw pyrolysis bio-oil using the method as described here [2], though common calibration standards, purchased from

Supelco, were used. As has been shown in previous round robin studies with bio-oil, GC-MS results showed relatively high variability. Here, 68% of quantified volatile compounds in bio-oil had variabilities of less than 20%. Variabilities of this order may seem high compared to physical characterization techniques such as ultimate analysis, but they are comparable to published standard methods using GC-MS, such as EPA method 8270D.

13. Quality Control

13.1 It is recommended that a representative bio-oil or standard solution be prepared as in 9.4.1 and analyzed with each sample set to provide a daily check standard. Results of this standard should be control charted to monitor for out of control results as defined as greater than 2 standard deviations of the mean of 10 replicate analyses conducted over the course of no less than three days.

13.2 Calibration verification should be carried out each day analyses are conducted. Calibration standard 5 should be injected before samples are analyzed and verified that concentrations determined do not differ from actual by greater than 15%.

13.3 Method repeatability should be assessed with triplicate analyses of one sample per day of analysis or each 10 samples prepared (as in 9.4.2). % RSD of all compounds quantified should be less than 5 %.

14. References

[1] ASTM D4307-99(2010), *Standard Practice for Preparation of Liquid Blends for Use as Analytical Standards*. 2010, ASTM International: West Conshohocken, PA.

[2] Ferrell, J.R. III; Olarte, M.V; Christensen, E.D.; Padmaperuma, A.B.; Connatser, R.M.; Stankovikj, F.; Meier, D.; Paasikallio, V. *Standardization of Chemical Analytical Techniques for Pyrolysis Bio-oil: History, Challenges, and Current Status of Methods*. Biofuels, Bioproducts & Biorefining 2015, submitted.