

# Determination of Biogenic Content by $^{14}\text{C}$ in Fuels using Liquid Scintillation Analysis

Laboratory Analytical Procedure

March 2022

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*for the*  
UNITED STATES DEPARTMENT OF ENERGY  
*under Contract DE-AC05-76RL01830*

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## **Suggested Citation**

Charles Doll, Andrew Plymale, Christopher Thompson, Matthew O'Hara and Mariefel V. Olarte. 2021. *Determination of Biogenic Content by  $^{14}\text{C}$  Measurement using Liquid Scintillation Counter*. Richland, WA: Pacific Northwest National Laboratory.

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Prepared for  
the U.S. Department of Energy  
under Contract DE-AC05-76RL01830

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## 1.0 Introduction

- 1.1 Quantification of biogenic fuel content in fuel blendstock is important at facilities that co-process biomass-derived liquids and fossil fuels as a mixture.  $^{14}\text{C}$  is an ideal tracer for biogenic fuel products, due to the depletion of  $^{14}\text{C}$  in fossil-based fuel, which allows direct liquid scintillation counting (LSC) to be used for quantitative measurement of  $^{14}\text{C}$  in low-level biogenic gasoline, jet fuel, and diesel range hydrocarbons in fuel blendstock to determine the biogenic content.
- 1.2 It is feasible to accurately determine the biogenic fraction of blended fuels containing as low as 1 wt% biogenic fuel using the direct LSC method, in which a fuel sample is directly mixed with LSC cocktail and analyzed using either Perkin-Elmer Quantulus or TriCarb instruments. The TriCarb offers the optional bismuth germanium oxide (BGO) guard, which is required to make accurate, low-level fuel analysis possible. The Quantulus and TriCarb instruments were found to be comparable for low-level detection of  $^{14}\text{C}$  in blended samples, suggesting that some instrument flexibility may be available to potential users [1].
- 1.3 This procedure covers the determination of the percent  $^{14}\text{C}$  in biogenic gasoline, jet fuel, and diesel range hydrocarbons prepared from distillation of hydrotreated pyrolysis oils and blended with fossil derived hydrocarbons (e.g. fossil diesel, gasoline, jet, or toluene). Either an internal  $^{14}\text{C}$  standard or a color quench curve is used to correct for potential signal quenching effects. Recent publications provide some background of direct LSC methods used for determination of biogenic fuel content [1,2].

## 2.0 Scope

- 2.1 This procedure has been developed and optimized for the quantification of biofuel content in mixed biogenic fuel:fossil fuel samples which contain raw pyrolysis gasoline, jet, and diesel bio-oil fractions.

## 3.0 Terminology

- 3.1 *Biogenic fuel* – fuel produced from biological sources, such as biomass
- 3.2 *Bio-oil* – The crude liquid product of converting lignocellulosic biomass into a liquid via pyrolysis. Another term, biocrude, is typically associated with hydrothermal liquefaction-derived liquid.
- 3.3 *LSC* – Liquid Scintillation Counting (Counter)
- 3.4 *tSIE* – transformed Spectral Index of External Standard
- 3.5 *dpm* – disintegrations per minute
- 3.6 *cpm* – counts per minute
- 3.7 *AEC* – automatic efficiency correction
- 3.9 *AMS* – Accelerator Mass Spectrometer
- 3.10 *pMC* – percent modern carbon

3.11 *LLCM* – Low-level counting mode

## 4.0 Interferences

4.1 Color or chemical quenching effects have the potential to decrease the measured dpm in a sample. Quench correction methods are incorporated to account for possible quenching effects on the results. In addition, chemiluminescence may increase count rates, such that counting windows may need to be optimized to eliminate chemiluminescence counts.

## 5.0 Apparatus

5.1 Analytical balance, accurate to 0.1 mg

5.2 Liquid Scintillation Spectrometer Instrumentation:

5.2.1 Perkin Elmer Quantulus 1220 Low Level Liquid Scintillation Spectrometer (or equivalent)

5.2.1.1 Count time – 1, 2, and 5 hours (un-spiked samples); 1 hour (spiked samples); and 0.5 hours (color quench standards)

5.2.1.2 Counts – No Limit

5.2.1.3 CUCNTS – No Limit

5.2.1.4 MCW – 1

5.2.1.5 Rep – 1

5.2.1.6 ST – No

5.2.1.7 Configuration – <sup>14</sup>C High Energy

5.2.1.8 Send Spectra – 11

5.2.1.9 PAC – 1

5.2.1.10 Coincidence bias – High

5.2.1.11 Channels – 50-650

5.2.1.12 STD – Yes

5.2.1.13 STIME – 1:00

5.2.2 Perkin-Elmer TriCarb Liquid Scintillation Analyzer with bismuth germanium oxide (BGO) guard (or equivalent)

5.2.2.1 Count times – 1, 2, and 5 hours (un-spiked samples); 1 hour (spiked samples); and 0.5 hours (color quench standards)

5.2.2.2 Counting modes – Super low-level mode (for cpm values for un-spiked and spiked samples and color quench standards, dpm values using color quench curve) and normal mode (for dpm values for spiked samples and color quench standards)

5.2.2.3 Assay types – cpm (un-spiked and spiked samples, color quench standards) and dpm (spiked samples, color quench standards)

- 5.2.2.4 Count corrections – antistatic
  - 5.2.2.5 Quench indicator – SIS (unspiked samples), tSIE/AEC (spiked samples and color quench standards)
  - 5.2.2.6 Counting window – 0 – 156 keV (with automatic window optimization using tSIE/AEC)
- 5.3 Elementar Vario Macro cube (for CHNS) or equivalent instrument. (or equivalent instrument)
- 5.4 Radiological workspace (e.g. radiological posted hood)

## 6.0 Reagents and Materials

- 6.1 Reagents
- 6.1.1 Standard:  $^{14}\text{C}$ -n-hexadecane (National Institute of Standards & Technology)
  - 6.2.2 Ultima Gold F LSC cocktail (Perkin Elmer) or equivalent
  - 6.2.3 Toluene ( $\geq 99.5\%$ , petroleum/fossil based)
  - 6.2.4 Fossil fuel for standard curve (close to the same boiling point range and carbon wt% as fuel being analyzed if known)
  - 6.2.5 Biogenic fuel for standard curve (close to the same boiling point range and carbon wt% as fuel being analyzed if known)
  - 6.2.6 Sudan IV (Solvent red 24, CAS [85-83-6]) (**Only required when preparing color quench curve**)
- 6.2 Materials
- 6.2.1 Glassware
    - 6.2.1.1 Three 10mL volumetric flasks
    - 6.2.1.2 1, 5, 10, and 25mL graduated disposable glass pipettes
    - 6.2.1.3 40 $\mu\text{l}$  glass microcapillary pipettes
    - 6.2.1.4 20mL glass scintillation vials (**foil or Teflon-lined caps**)

## 7.0 ES&H Considerations and Hazards

- 7.1 Follow all applicable chemical and radiochemical handling procedures.

## 8.0 Sampling, Test Specimens, and Test Units

- 8.1 Care must be taken to ensure that a representative sample is taken for analysis. Shake biogenic fuel at ambient temperature as vigorously as possible.
- 8.2 Samples should be stored in the dark following addition of LSC cocktail.
- 8.3 Samples should be allowed to equilibrate in the dark on the instrument for at least 5 hours to reduce potential chemiluminescence effect on LSC counting.



## 9.0 Analytical Procedure

### 9.1 Spiking Standard Preparation

#### **(Only required when performing quench correction with internal spike)**

- 9.1.1 Prepare a Stock Standard solution of approximately 2500 dpm/ $\mu$ l  $^{14}$ C by diluting the NIST standard with toluene. Example calculation for determination of stock standard concentration:

$$\text{Stock Standard Concentration } \left( \frac{\text{dpm}}{\mu\text{l}} \right) = \text{Concentration of NIST standard } \frac{\text{Bq}}{\text{ml}} \times 60 \frac{\text{dpm}}{\text{Bq}} \times 1 \frac{\text{ml}}{1000\mu\text{l}}$$

Weights for each addition should be tabulated for concentration calculations.

#### 9.1.2 Spiking Standard Dilution for Spiking Solution

- 9.1.2.1 Spiking Standard A. Perform a 1/10 dilution by pipetting 1 mL of the 2500 dpm/ $\mu$ l standard into a 10 mL volumetric flask and diluting to the mark with toluene. The weight for each addition should be tabulated for concentration calculations. Target concentration: 250 dpm/ $\mu$ l.
- 9.1.2.2 Spiking Standard B. Perform a 1/10 dilution by pipetting 1 mL of Standard A into a 10 mL volumetric flask and diluting to the mark with toluene. The weight for each addition should be tabulated for concentration calculations. Target concentration: 25 dpm/ $\mu$ l.
- 9.1.2.3 Spiking Standard C. Perform a 1/10 dilution by pipetting 1 mL of Standard B into a 10 mL volumetric flask and diluting to the mark with toluene. The weight for each addition should be tabulated for concentration calculations. Target concentration: 2.5 dpm/ $\mu$ l.

### 9.2 Standard Curve Preparation

- 9.2.1 Label glass LSC vial caps
- 9.2.2 Pre-weigh the vials and record weights
- 9.2.3 Using graduated pipettes, prepare 20% biogenic fuel dilutions of fuel to be used for standard curve(s) (e.g. gasoline, jet, and diesel diluted in fossil fuel or toluene). Record weights (See Table 1 example).
- 9.2.4 Using graduated pipettes, pipette 20% biofuel stock from above into LSC vials – use sample volumes from Table 2 (found in section 10) to prepare mixed samples by diluting the 20% biofuel standard with fossil fuel. Record the weight of the biofuel and vial.
- 9.2.5 Using graduated pipettes, pipette fossil fuel into LSC vials – use sample volumes from Table 2 to prepare mixed samples. Record the weight of the fuel and vial.
- 9.2.6 Finally, pipette 13 mL of Ultima Gold F into the LSC vials containing the mixed samples.
- 9.2.7 Thoroughly mix the contents of the vials.

### 9.3 Sample Preparation

- 9.3.1 Label glass LSC vial caps.

- 9.3.2 Pre-weigh the vials and record weight.
- 9.3.3 Pipette 7 mL of fuel sample into vial (prepare in triplicate). Record the weight of the fuel and vial.
- 9.3.4 Pipette 13 mL of Ultima Gold F into the LSC vials containing the samples. Record the weight of the sample and vial.
- 9.3.5 Thoroughly mix the contents of the vials.
- 9.4 Prepare LSC blank samples.
  - 9.4.1 Label glass LSC vial caps.
  - 9.4.2 Add 20 ml of Ultima Gold F to each vial (prepare in duplicate).
- 9.5 Analyze the un-spiked standards and samples by LSC.
  - 9.5.1 Use standard counting sample parameters from section 6.1.

**Note: Steps 9.6 and 9.7 are only required when performing quench correction with internal spike.**

- 9.6 After counting the un-spiked standards and samples, spike each standard and sample with 40 $\mu$ l of Standard C: 2.5dpm/ $\mu$ l (~100 dpm total spike) and mix well. These are the spiked standards and samples.
- 9.7 Analyze the spiked standards and samples by LSC.
  - 9.7.1 Use standard counting sample parameters from section 6.1.
- 9.8 Elemental analysis to determine Total Carbon wt% of standards and samples.
  - 9.8.1 Perform analysis of carbon with an Elementar Vario Macro cube (for CHNS) or equivalent instrument.
  - 9.8.2 For Elementar Vario Macro cube:
    - 9.8.2.1 Weigh approximately 10-30 $\mu$ L of sample into a tin capsule.
    - 9.8.2.2 Flush the capsule with helium and then seal to avoid ambient air inclusion.
    - 9.8.2.3 The combustion tube is heated to 1150°C and the reduction tube to 850°C.
    - 9.8.2.4 Measure a minimum of two samples and report the average.

**Note: Step 9.9 is only required when performing color quench curve correction.**

- 9.9 Preparation of color quench curve standards.
  - 9.9.1 Add 13 ml of Ultima Gold F cocktail to ~15 LSC vials (no need to weigh).
  - 9.9.2 Spike each vial with an exact amount of <sup>14</sup>C hexadecane standard to give 10,000-20,000 dpm, with sample-to-sample precision as close as possible.
  - 9.9.3 Assay for 30 minutes on LSC. If dpm protocol (or chemical quench curve) is available, determine dpm value for each vial (normal mode on TriCarb). If dpm

protocol (or chemical quench curve) is not available, then aliquots in step 9.9.2 above should be weighed, so that an exact dpm value is known for each vial.

- 9.9.4 Calculate median dpm of the ~15 vials, and use the ~12 vials containing 13 ml of cocktail that are closest to the median to prepare the quench standards. The ~3 extra vials containing 13 ml of cocktail can be used for initial quench testing.
- 9.9.5 Weigh Sudan IV and mix with toluene to prepare a Primary Sudan IV stock of ~0.01% Sudan IV in toluene. **Note:** Sudan IV is a carcinogen, take appropriate precautions when handling.
- 9.9.6 Dilute the Primary Sudan IV stock 1/10 in toluene to give ~0.001% Sudan IV. For initial testing, add 7 ml of 0.001% Sudan IV to one of the extra three spiked vials containing 13 ml of cocktail, along with one vial of 13 ml cocktail + 7 ml of toluene.
- 9.9.7 Assay the two vials (13 ml cocktail + 7 mL toluene, and 13 ml cocktail + 7 ml of 0.024% Sudan IV in toluene) on the TriCarb to determine the tSIE value for each vial. The vial without Sudan IV should have a tSIE value of ~700 or above. The vial with Sudan IV should have a tSIE of 25-50. If the tSIE value of the latter is outside the range 25-50, then make either a more dilute or a more concentrated stock, to give a tSIE value of within the desired range. Add 7 ml of new stock to the 3rd extra 13-ml cocktail vial and re-assay to confirm tSIE value.
- 9.9.8 Make further dilutions of Sudan IV in toluene to give approximate dilutions as shown in Table 1, with approximate tSIE values shown.

Table 1. Example color quench curve standards

vial #	Sudan IV conc. (% w/w)	unquenched* dpm	tSIE (normal mode)	tSIE (low-level mode)	quenched cpm (normal mode)	quenched cpm (low-level mode)	efficiency (normal mode)	efficiency (low-level mode)
1	0	17295	732	744	16390	11616	94.02	66.64
2	2.53E-10	17580	692	709	16598	11848	95.22	67.96
3	5.05E-08	17623	477	477	16151	11905	92.65	68.29
4	1.01E-06	17513	334	335	15624	11658	89.63	66.88
5	1.01E-05	17442	206	208	14659	11209	84.09	64.3
6	5.39E-05	17545	164	167	13874	10869	79.59	62.35
7	2.16E-04	17102	108	108	11430	9267	65.57	53.16
8	5.75E-04	17311	79	79	9408	7889	53.97	45.26
9	1.15E-03	17553	33	34	3194	2895	18.32	16.61

\*Before addition of Sudan IV and toluene; Average unquenched dpm of 17533, standard deviation of 171 dpm, median of 17513 dpm.

- 9.9.9 Add 7 ml of each Sudan IV dilution to each of 10 vials containing 13 ml of cocktail, and to one vial containing 13 ml cocktail, add 7 ml of toluene.
- 9.9.10 Re-assay vials to determine quenched cpm values (for TriCarb use both normal and low-level counting modes), and use these values to determine color quench standard curve efficiency for each quenched standard, where:

$$\text{Color Quench Standard Curve Efficiency} = \frac{\text{Quenched CPM}}{\text{unquenched DPM}} \times 100$$

## 10.0 Results

10.1 The following tables can be used as a guide to record data.

Table 2. 20% sample weights

Name/Date	% Biogenic fuel	Vial (g)	+Fossil Fuel (g)	+ 20% Biogenic Fuel (g)
	20			
	20			
	20			

Table 3. Mixed standards pipetting volumes

Standard	% Biogenic fuel	Fossil fuel (ml)	20% Biogenic Fuel (ml)
S0	0	7	0
S1	1	6.6	0.4
S2	2	6.3	0.7
S3	3	6.0	1.0
S5	5	5.2	1.8
S10	10	3.5	3.5

Table 4. Mixed standard weights (standard curve)

Standard	% Biogenic fuel	Vial (g)	+fossil diesel (g)	+Biogenic fuel (g)	+Cocktail (g)
S0	0				
S1	1				
S2	2				
S3	3				
S5	5				
S10	10				

Table 5. Sample weights

Name/Date	Vial (g)	+fossil diesel (g)	+Biogenic fuel (g)	+Cocktail (g)

## 11.0 Calculations

11.1 There are two options for calculation of the percent biogenic fuel and pMC in the samples. The internal spike quench correction is theoretically most accurate under all conditions, but requires spiking the samples with  $^{14}\text{C}$  standards and additional instrument count time. Quench curve correction has the advantage of requiring fewer steps and is sufficiently accurate for most situations.

11.2 Internal spike quench correction and determination of percent biogenic fuel.

11.2.1 Calculate the % Quenching.

$$\% \text{ Quenching} = 100 - \frac{\text{Spiked Sample CPM} - \text{Un-spiked Sample CPM}}{\text{Spiked LSC blank CPM} - \text{Un-spiked LSC Blank CPM}} \times 100$$

11.2.2 Calculate the Quenching Factor.

$$\text{Quenching Factor} = \frac{100 - \% \text{ Quenching}}{100}$$

11.2.3 Calculate Quench Corrected CPM.

$$\text{Quench Corrected CPM} = \frac{\text{Spiked Sample CPM}}{\text{Quenching Factor}}$$

11.2.4 Determine the Quench Corrected % Biogenic fuel.

11.1.4.1 Plot Quench Corrected CPM values of standard curve samples vs Percent Biogenic Fuel (calculated gravimetrically) and determine the linear equation.

11.1.4.2 Determine the Quench Corrected % Biogenic fuel by applying the linear equation to the Sample Quench Corrected CPM.

11.2.5 Determine pMC (**needed if the carbon content of the fuel to prepare the standard curve and samples differ significantly**)

$$pMC = \text{Quench Corrected \% Biogenic Fuel} \times \frac{\text{Sample Total Carbon wt\%}}{\text{Standard Total Carbon wt\%}}$$

11.3 Quench curve correction and determination of % Biogenic Fuel (tSIE)

11.3.1 Calculate Efficiency for standards

$$\text{Color Quench Curve Efficiency} = \frac{\text{Quenched CPM}}{\text{Unquenched DPM}} \times 100$$

11.3.2 Plot the color quench curve efficiency vs. tSIE to obtain quench curve and determine the best fit equation for the plotted data. Alternatively, the quench standards can be run on a quench standard protocol on the TriCarb in low-level counting mode (LLCM), so that the instrument can automatically calculate the efficiency for a given tSIE value.

11.3.3 Determine the Quench Curve Efficiency by applying the equation found in step 11.3.2 along with the tSIE values for the standards and samples.

11.3.4 Calculate Efficiency Corrected DPM for standards and samples

$$\text{Efficiency Corrected DPM} = \frac{\text{measured CPM}}{\text{Quench Curve Efficiency}}$$

11.3.5 Plot the Efficiency Corrected DPM standards vs. the % Biogenic Fuel (calculated gravimetrically) and determine the linear equation.

11.3.6 Determine the Quench Curve Corrected % Biogenic fuel by applying the linear equation to the Efficiency Corrected DPM Corrected CPM.

11.3.7 Determine pMC (**needed if the carbon content of the fuel to prepare the standard curve and samples differ significantly**)

$$pMC = \text{Quench Curve Corrected \% Biogenic Fuel} \times \frac{\text{Sample Total Carbon wt\%}}{\text{Standard Total Carbon wt\%}}$$

## 12.0 Report Format

- 12.1 Report the percent biofuel content determined for each sample along with the average biogenic fuel content and standard deviation for replicate samples.
- 12.2 Report the pMC values for each sample along with the average pMC values and standard deviation for replicate samples.

## 13.0 Quality Control

- 13.1 Recommended Significant Figures: Report results with one decimal place.
- 13.2 Replicates: Run all samples in triplicate.

## 14.0 References

[1] Charles G. Doll, Andrew E. Plymale, Alan Cooper, Igor Kutnyakov, Marie Swita, Teresa Lemmon, Mariefel V. Olarte, Huamin Wang (2021) Determination of low-level biogenic gasoline, jet fuel, and diesel in blends using the direct liquid scintillation counting method for <sup>14</sup>C content. Fuel, Volume 291, 120084, ISSN 0016-2361, <https://doi.org/10.1016/j.fuel.2020.120084>.

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