STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Gas Chromatography Calibration for Ethanol

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APPROVALS: Process Change Committee **DATE: December 12, 2012**

A. Scope

This procedure describes how to make an ethanol calibration curve for the determination of ethanol concentration by gas chromatography.

B. Safety and Training Requirements

Refer to UF lab safety policies and review the Material Safety Data Sheets (MSDS) for each material listed in section D below before starting any process work.

Review the location of fire extinguishers, fire blankets, safety showers, spill cleanup equipment and protective gear before beginning any process work.

During operations in the laboratory, the following safety gear will be utilized at all times:

- Lab Coat
- Safety Goggles
- Protective Gloves (nitrile, neoprene)

C. Related Documents and SOPs

1. Agilent Technologies 6890N Network GC System operating manual.

D. Preparation/Materials/Equipment

1. Agilent Technologies 6890N Network GC System G1530N (autosampler with 150 sample capacity, wide bore column and split injection)
2. GC Column JW Scientific 19095P-QO3E HP-PLOT/Q
3. National Scientific Target KP Vials (C4000-1)
4. National Scientific DP Vial Caps (C4000-51G, PTEF/RR SEPTA, 100/PK, 100/CS)
5. Pipettor (200-1000 µL range)

6. Pipette tips (1000 µL)
7. 1-Propanol (Fisher-Scientific, A414-4, 4 L)
8. Absolute Ethanol 99.5% (ARCOS, #61509-0040)
9. DI water
10. Volumetric flasks
11. Compressed hydrogen cylinder
12. Compressed air cylinder
13. Compressed helium cylinder

E. **Detailed Procedure**

1. Prepare a solution of 1-propanol at 2% (w/v) with DI water in a volumetric flask and storage it at 4 °C in the refrigerator.
2. Prepare 5 different concentrations of ethanol (10, 20, 30 and 40 g/L) in DI water using Abosulte Ethanol in volumetric flasks. Use all solutions the same day they are prepared.
3. In order to make the ethanol calibration curve, prepare 5 different samples by pipeting 300 µL of the 2% (w/v) 1-propanol solution and 300 µL of the different solutions of ethanol (0, 10, 20, 30 and 40 g/L) into a National Scientific Target KP Vial using a 1 mL pipettor and tips (200-1000 µL range). Label the glass vials with the ethanol concentration used.
4. Seal the vials with the appropriate screw-caps.
5. Turn on the GC computer and open the Chemstation software in the Online mode.
6. Turn on the Gas Chromatograph (GC) by;
    a. Open the main valves of the different gas cylinders (compressed hydrogen, compressed air and compressed helium).
    b. Assure that the line pressures of the gasses are 40 PSI for Hydrogen; 60 PSI for Air; 80 PSI for Helium
    c. Assure that there is sufficient gas in each of the cylinders by checking the cylinder pressure gauge.
    d. Turn on the GC using the power button at the bottom right hand of the GC or using the Chemstation software.
7. After 10 minutes, make sure in the Chemstation software that the gas flow rates are 25 mL/min for helium, 45 mL/min for hydrogen, and 450 mL/min for air.

a. If the GC flame does not ignite, the software will give a warning and the GC will not start properly. Contact the shift supervisor.

8. In the Online mode, create a new sequence table (with the sequence function) with the different ethanol concentrations (0, 10, 20, 30 and 40 g/L).
    a. Assure the method ETOH.M is loaded in the software.
9. Save the sequence in a subdirectory in C:/Chem32 with a new name (use the "save as" option, not the save button).
10. Mix all the samples of the ethanol calibration curve by inversion for 3 seconds and place them in the GC sample holder in the same order as the sequence table.
11. Make sure the wash bottle contains DI water and the waste bottles are empty in the GC carousel.
12. Make sure that the temperature in the oven is 350 °C and in the injection chamber is 250 °C
13. Press the RUN button in the Online mode.
14. After all the samples are processed in the GC system, open the Chemstation software in the Offline mode and open the sequence to be analyzed.
15. Read the ethanol and 1-propanol area on the GC chromatogram.
16. Divide the ethanol area by the 1-propanol area and plot the result as a function of ethanol concentration used in each sample.
17. Create a linear regression line from the plot.
    a. If the R-squared value of the linear regression is lower than 0.95, repeat the calibration.
    b. The slope will be used to determine the ethanol concentration in grams per liter of all samples analyzed.
18. Remove all vials from the GC sample holder and turn off the GC system.
19. Close the valves of the different gas cylinders.

F. Data Archival and Analysis

Record all the ethanol and 1-propanol areas, the date, time and ethanol concentrations used to make the ethanol calibration curve in the laboratory notebook.