CO_2-CH_4-N_2O by Gas Chromotography (Shimadzu) Version 1.0

I. Overview

Gas Chromotography (GC) separates component gases that are then detected by a detector. These components are then quantified using a standard curve. The Shimadzu Specialized GC-2014 Green House Gas Analysis gas chromatograph system equipped with the AOC 6000 auto sampler and FID and ECD detectors and Shimadzu Lab Solutions software enables the simultaneous qualitative and quantitative analysis of three major greenhouse gases: methane(CH_4), carbon dioxide(CO_2), and nitrous oxide (N_2O). The Flame Ionization Detector (FID) interacts with analytes eluting from the GC column. The detector produces a current that changes in proportion to the concentration of the analyte. The FID detects any molecule with a hydrogen-carbon bond. The analyte is ionized in a flame produced by hydrogen and air. Ions travel across and electric potential toward an electode, the current is converted to a voltage which is filtered and amplified. The FID is equipped with a methanizer that is necessary for detection of low concentrations of carbon dioxide (CO_2) and carbon monoxide (CO) by converting these compound to methane before detection. The Electron Capture Detector (ECD) detector emits electrons that ionize the make-up gas molecules creating a stable electron cloud. The ECD maintains a constant current equal to the cloud by applying periodic pulses. When a gas is injected, electronegative compounds enter the ECD cell and combine with free electrons in the cloud. In response, the ECD increases it's pulse rate to maintain a constant current. The ECD is used to detect N_2O.

Standard injections of known concentration and volume and their peak areas are used to determine the concentration of unknown gases by measuring the area of the unknown and then relating these areas to a regression obtained from the standard injections.

II. Equipment

A. Shimadzu GC 2014 with AOC 6000 auto injector. Detectors are flame Ionization Detector (FID) and Electron Capture Detector (ECD). Detector response is calibrated using Calibration Standards prepared for each instrument run.
B. LabSolutions Chromatography Integration Software controls the gas chromatorgraph and the auto sampler
C. Make-up gas – Nitrogen Ultra High Purity (UHP)
D. Carrier gas – Nitrogen UHP
E. FID Detector gas- HydrogenUHP, Air UHP
F. Septa Septa, GC, Long Life Septum, 450C, Blue, 20/PK-Septa, GC, Long Life Septum, 450C, Blue

G. Column – Hayesep D 100/120. 10’ x 1/8”x 0.085”. Stainless steel.
H. Gas standard – Custom Certified Stock Gas Standard-
    A. Mesa Specialty Gases & Equipment:
        1. CO_2 (5000ppm), CH_4 (100 ppm), N_2O (5ppm)
        2. Referred to as Mesa Stock Standard 2024-27
I. SupelTM Inert Foil Gas Sampling Bags p/n 30226-U
J. Evacuated Labco Exetainer 12mL Vials, flat bottom with DW white cap
    A. See NEON Sample Container Preparation Protocol
K. Gas tight disposable syringes
    A. 2x 20mL
    B. 2 x 10mL
    C. 2 x 3 mL

III. Safety

A. Make sure all the connections are leak free. To check for leaks, squirt soapy water on each connection. There will be bubbles formed if the connection leaks. Tighten connections as needed.
B. Do not open the instrument while running.
C. Gas cylinders must be secured at all times when protective caps are off.

IV. Gas Standard Dilutions for Calibration Standards and Check standards.
A. Calibration standards and Check Standards should be made at the same time to minimize error.
B. Mesa Stock from canister
C. UHP Nitrogen from gas cylinder

A. Dilutions are made in 12mL evacuated exetainers overfilled to 18~~2~~L
    a. 8 evacuated exetainers are needed for calibration levels
    b. 6 evacuated exetainers are needed for check standards
    c. 4 evacuated exetainers are needed for N2 method blanks
B. Inert Foil Gas Sampling bags
    a. Fill before each set of standards are made
        i. Open valve on foil bag by twisting left
        ii. Open valve on canister or cylinder to flush tubing
        iii. Attach nozzle on foil bag to tubing
        iv. Fill until taught but do not over fill
        v. Close valve on bag before removing from tubing
        vi. Close valve on cylinder or canister

C. Using gas tight disposable syringes create Calibration standards and Check standards according to Table 1.
    a. Have a different syringe each for nitrogen and Mesa stock standard
        i. 1x20mL for N2 and Mesa each
        ii. 1x10mL for N2 and Mesa each
        iii. 1x3mL syringe for N2 and Mesa each

D. Create 2 exetainers at the Calibration Level 2. One will be run as Calibration level 1 run at 0.4mL instead of 1mL

Table 1. CALIBRATION AND CHECK STANDARDS

CAL LEVEL	STD NAME	**MESA STOCK STANDARD 24-27 mL (syringe size)**	**N2 ML (syringe size)**	C02 CONC (PPMV)	CH4 CONC (PPMV)	N20 CONC (PPMV)
Level 1	MESA 0.01	**CAL LEVEL 2**		50	1	0.05
Level 2	MESA 0.025	**0.5 (3mL)**	**19.5(20m)**	125	2.5	0.125
Level 3	MESA 0.05	**1(3mL)**	**19(20mL)**	250	5	0.25
Level 4	MESA 0.1	**2 (3mL)**	**18(20mL)**	500	10	0.5
Level 5	MESA 0.25	**5(10mL)**	**15(20mL)**	1250	25	1.25
Level 6	MESA 0.5	**10(10mL)**	**10(10mL)**	2500	50	2.5
Level 7	MESA 0.75	**15(20mL)**	**5(10mL)**	3750	75	3.75
Level 8	MESA 1.0	**20(20mL)**	------	5000	100	5

E. Create Method Blanks to be run twice each during the run
   a. 20mL UHP Nitrogen
   b. Run before and after Calibration Standards
   c. Run one after every 10 samples

V. Preparing the GC-2014 and AOC 6000 auto sampler
Before turning on the gases
   A. Turn on the Instrument, bottom right corner
      A. It will run diagnostics
   B. Check to see if the septa needs to be changed
      A. Septa needs to be changed every 100-150 samples
      B. 'Diag' button on instrument panel-Analysis Counter
      C. Replace septum if needed-see Replace septa
      D. Reset counter on instrument panel
         1. Reset both septa and insert count
   C. Check Gas pressure at tanks
      A. If the main tank pressure is less than 300psi, the tank must be changed
         1. Only change gas tanks when instrument is not running

VI. Starting the Instrument
   A. Check for instrument computer to see the AOC icon in the bottom right corner is green
      A. If it is not green, the AOC is not on, turn on the AOC at the switch box
      B. Turn on the instrument gases

1. THE GASES MUST BE TURNED ON IN ORDER
    a. Zero Air-60psi
    b. UHP Hydrogen-100psi
    c. UHP Nitrogen-80psi
2. If the gases are not turned on in order, the flame will not stay lit on the FID
    a. If this occurs you must wait for the gases to go back to zero on the instrument pressure gauges and start again
3. Check instrument pressure gauges above the instrument
    a. Wait for all gases to come to the marked pressure

C. Open Labsolutions Software
1. Double click on Lab Solutions icon on desktop
2. Select “Instruments” icon
3. Double click DESKTOP-F3N60CM Instrument 1

D. Open Current Method
1. File>Open Method File
    a. NEON_GHG_YEARDAYMONTH
2. Click “System On” on side panel
3. Upload method to instrument
    a. Click Upload Parameters button
        i. Bottom half of screen

E. Wait until instrument is ready
1. In the upper right corner of the instrument screen
    a. AOC ready
    b. Instrument ready
2. Wait a minimum of 1 hour for the instrument to equilibrate before starting a batch
3. FID flame will ignite when the appropriate temperature is reached
    a. If the FID flame does not stay lit, shut down
        i. Select Instrument>shutdown
        ii. Wait for temperatures to all be 50 degrees or less
        iii. turn off instrument and wait for all gauges are at zero pressure to restart.

VII. Create Batch Tables- Figure 1.

A. Open most recent Sample Batch Table
    A. File>open Batch

B. Save Batch run as: NEON_GHG_SETNUMBER_YYYYDDMM

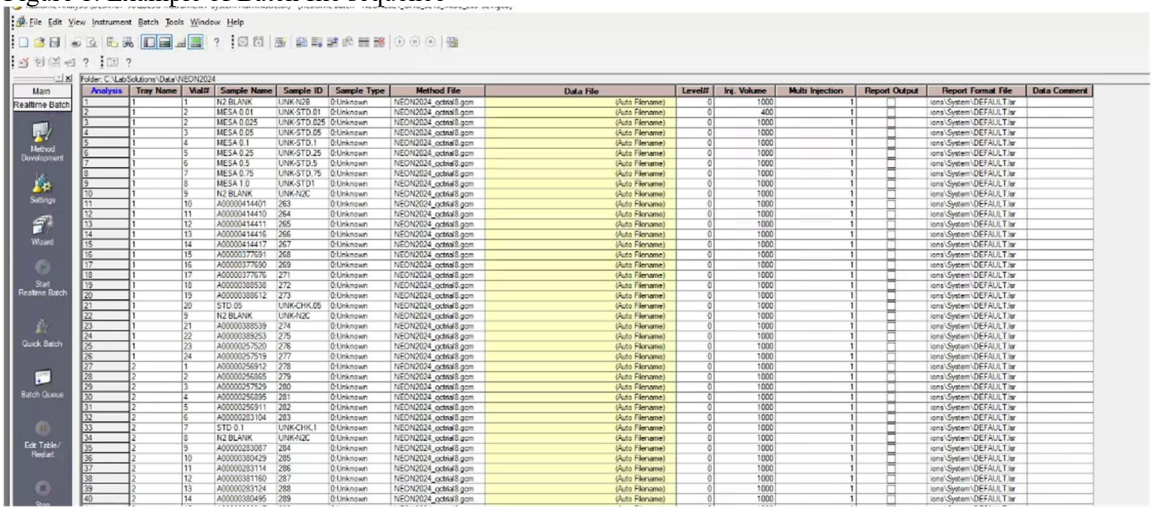
C. Update sample names
    A. Open the updated USUABL_NEONSDG_SAMPLEID excel spreadsheet

1. Desktop file
2. Copy/paste
    a. Sample name is NEON barcode number
    b. Sample ID is number assigned by USUABL Lab

D. All Standards and samples must be entered as "Unknown" in the Sample type column
E. **Calibration level 1 (Mesa 0.025)** volume injected should be set at 400uL
    A. All other standards and samples should be 1000uL
F. Update method for all lines in the batch table
    A. Click on drop down box in the method column of the first sample
        1. Select the method with the most recent date
        2. Copy and paste the updated method and fill down for all the batch lines

G. Run up to 62 samples per run
H. Run a check standard after every 10 samples
    A. Create new standards for check standards (do not refer to vials already run)
        1. Choose from Level 2-8
            a. Midlevel
        2. Run method blank every 10 samples
            a. 20mLUHP Nitrogen
            b. Ok to rerun from previous vials in run
            c. Analyte concentrations in the method blank should be less than the method detection limits
    B. Save the batch

Figure 1. Example of Batch file sequence



VIII. Load Samples
- A. Load exetainers into vial trays
  - A. 3 trays holding 24 vials each
    1. Tray 1 is closest to the instrument, Tray 3 farthest out
  - B. Indicate which tray and slot number on sequence run
- B. Run a Method blank (UHP Nitrogen before and after calibration standards and after every 10 samples. Detected levels for all analytes should be less than the analyte MDL. If there are one or more peaks detected above the detection limit, repeat the blank injection. If peaks are still detected, cancel run, shut down instrument, change septum and check system for leaks before starting again.
- C. Samples are injected at 1 mL unless previously run and concentrations exceed the calibration limits
  - A. If sample concentration is too high, inject a smaller volume according to level reported
- D. Inject a check standard at the beginning of each sample set, after every 10 unknown samples, and at the end of the. If in excess of $\pm$5%, the set must be re-run. Check standard percent deviation is calculated as :

  $\%dev = (C_{check}-C_{standard})/C_{standard} \times 100\%$
- E. If peak area or estimated concentration of a sample exceeds the highest level calibration standard,the sample must be re-run at a lower sample volume within 30 days.

IX. Start Batch Run
- A. Check that instrument is ready
  - A. Upper right corner, both instrument and AOC windows green
  - B. All temperatures are green
- B. Start Batch Run
  - A. Click 'Start Realtime Batch' button
    1. Methane Elutes at 3 minutes (FID)
    2. Carbon Dioxide Elutes at 6 minutes (FID)
    3. Nitrous Oxide Elutes at 7 minutes (ECD)
  - B. Run time is 8 minutes per sample

X. Check Calibration before letting all samples run
- A. After all initial calibration standards are run, double click on the first line in the batch table
  - A. Post-run application will open with that sample loaded
- B. In the left panel, click on Method tab
  - A. Open the method being run
    1. Calibration curve view will open
  - B. Save Method with the day's date (NEON_GHG_YEARDAYMONTH)
  - C. In the Data Files view the data files used in the calibration curve are displayed.

1. Right click on “Data Files” at the top of the files
    a. Click on “remove all”
        i. Takes old calibration standards out
2. In the right panel click on the Data files tab
    a. The calibration standards run for the current batch should be displayed
3. Drag and drop Calibration Standard files in from current run into appropriate levels

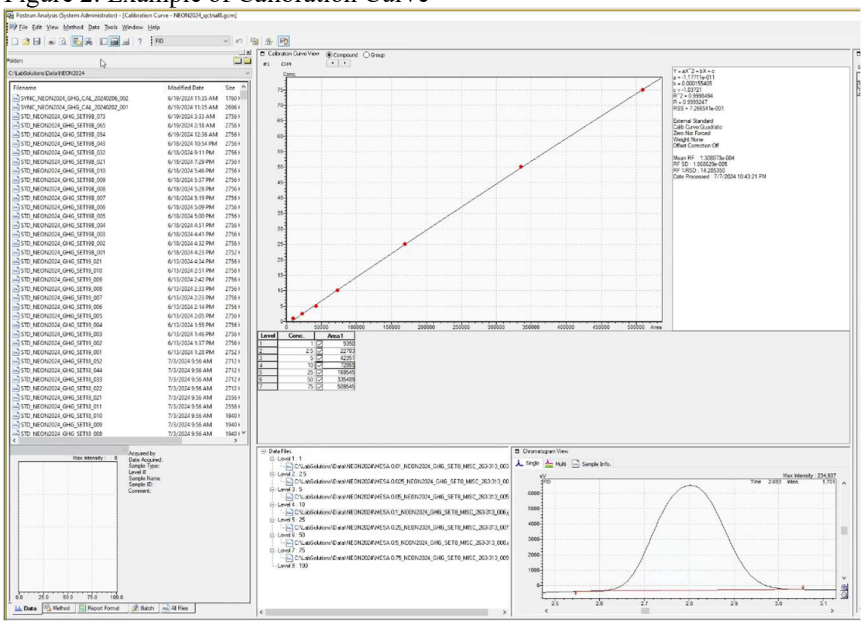
D. Check all analytes standard curve-3 curves
1. R^2 should be 0.99 or better
    a. Rerun if any of the curves are below 0.99
2. FID window-Toolbar drop down list
    a. Toggle arrows at top of Cal curve view
        i. Methane
        ii. Carbon Dioxide
3. ECD window-Toolbar drop down list
    a. Nitrous Oxide

E. If some standards are compromised, up to 3 levels can be removed to maximize linearity (min. 5 cal standards used for curve)
1. Do not remove the lowest and highest standards
2. If all the standards look good, leave them all in

F. If the R^2 for standards is lower than 0.99, stop the run and recreate standards

C. See above (VII C-F) for method blanks, check standards, and samples.

Figure 2. Example of Calibration Curve

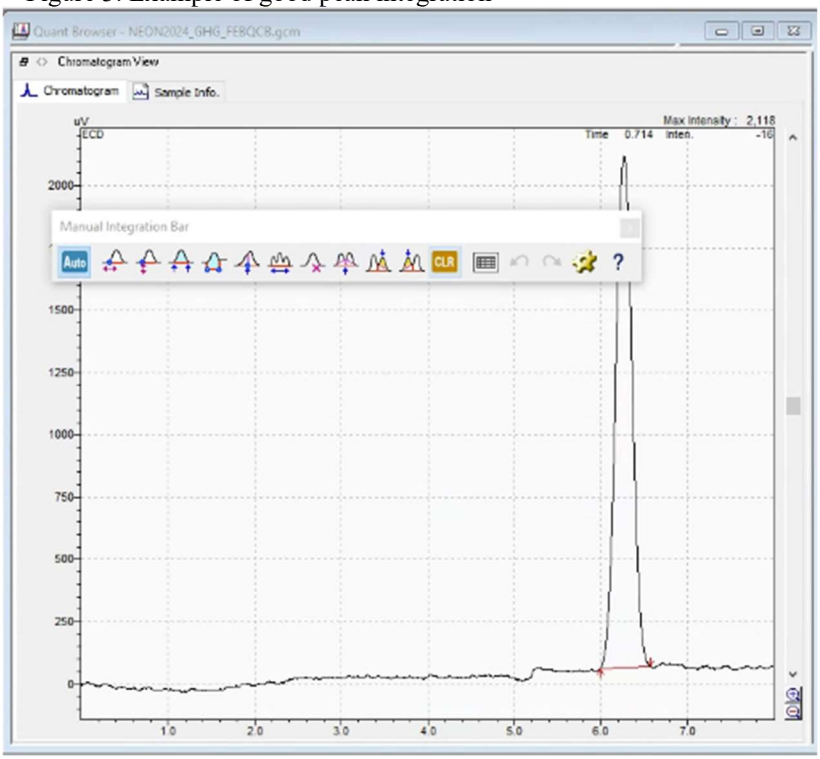


D. Shutting Down
    A. Open Instrument>Shutdown
        1. Instrument will start cooling down
        2. Do not turn off the gases until all temperatures are below 50 degrees. This will take at least an hour, usually 2.
    B. AFTER ALL TEMPERATURES ARE BELOW 50 DEGREES
        1. Turn off gases
        2. Turn off instrument
    C. Wait until the gas pressure on the instrument gauges have dropped to zero before starting a new run

XI. DATA ANALYSIS
    A. Data should be reviewed within 2 weeks of the sample run in order to identify any issues and rerun within a 30-day time period
    B. Be sure all other LabSolutions windows are closed
    C. In Lab Solutions Main Window
        A. Click on Postrun operations
            1. Open Postrun Tool
            2. PostRun Batch Window will open
    D. Click on “Batch” Tab
        A. Open Current Sample Batch
        B. Batch Sequence table will open
            1. Click on the Method column for the first sample
                a. “Select Method File” window will open
                b. Load the new method created with the calibration standards from the batch being analyzed from section X
                c. copy and paste the current method
                    i. This will change the method for all the files in the batch
            2. Save batch file
            3. In Main panel on far left click “Start Postrun Batch”
                a. This will process all the samples with the current calibration curve
                    i. This will take a minute
                    ii. It will not process any files that are open in other windows. You will get a prompt that it cannot process a sample. It is easiest if you close all other windows
        C. In LabSolutions Main window
            1. Select Postrun operations
                a. Open Browser Tool
            2. QuantBrowser Window will open
    E. Click on “Batch” Tab
        A. Open Current Sample Batch

1. This opens the files in the batch run
2. The calibration curve information is in the bottom right panel
3. A chromatogram view is displayed and integrations can be checked and updated for each file
    a. Peaks should be integrated the same for all samples
    b. Use manual integration toolbar to edit baseline
        i. Right click on chromatogram view
        ii. Select “manual integration bar”

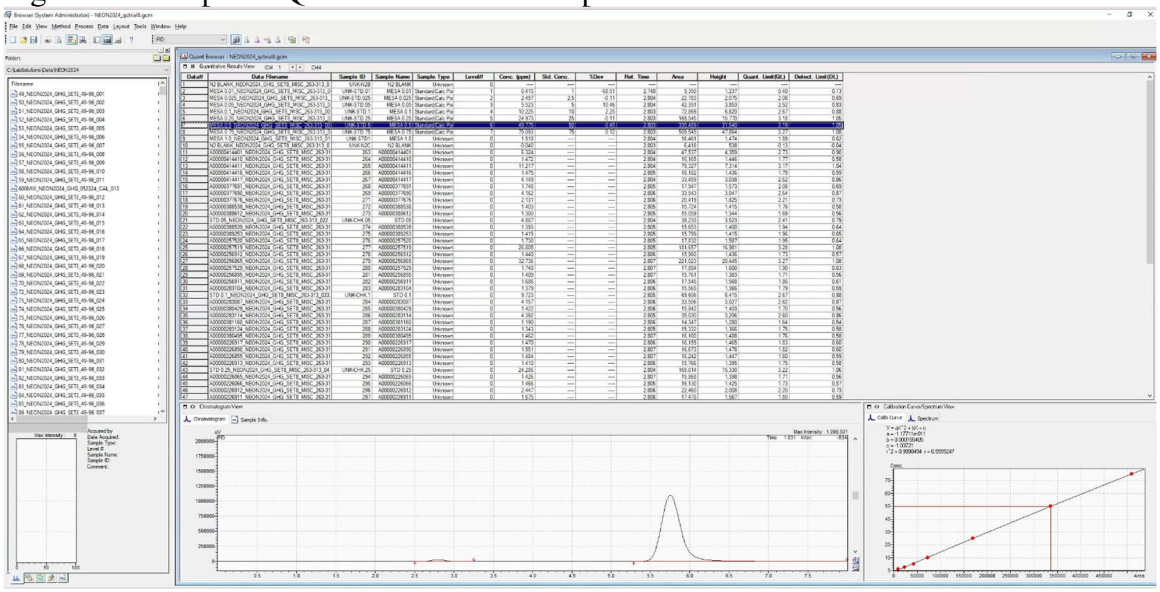
Figure 3. Example of good peak integration



4. Analytes with concentrations above the calibration range will need to be reanalyzed at a smaller volume according to the concentration reported
    a. ie if the methane is 150ppm (highest calibration level is 100ppmv), the injection volume should be halved to insure it is within range and above the mdl
5. Quantitative Result view displays for each analyte
    a. Data Filename
    b. Sample ID
    c. Conc (ppm)
    d. Expected concentration for standards

e. Quantitative Limit for each Sample (including standards)
f. Detection Limit for each Sample (including standards)
g. Accuracy (%) for Standards and Check standards

Figure 4. Example of QuantBrowser Data Report



F. Copy and paste Information for each analyte into Excel
    A. Drop down list at top for FID and ECD
        1. Toggle at top of Quantitative Results view for methane and carbon dioxide
G. Raw data on first tab produced by instrument
    A. All concentrations and QC information for all three analytes
H. Sample Concentrations on second tab produced by instrument
    A. Report concentrations only in ppmv for all three analytes per sample
I. QA/QC on third tab produced by instrument (for lab use only)
    A. All concentrations and QC information for calibration and check standards only for each analyte
    B. Average Quant Limit and Detection Limit for Calibration and check standard concentrations
    C. Calculate the RSD% for all check standards
        1. If the RSD% is above or below (+/-)5.0. the samples within bracketed by that check standard must be rerun
    D. Precision is calculated as the average of 6 repetitions of each standard level.

XII. Data Reporting for NEON

A. NEON requires data to be submitted via an ingest process on their web-based data portal. Per sample dissolved gas concentrations are reported using the perSample_Dissolved Gas template CSV file. In addition to the CO_2, CH_4, and N_2O concentrations determined for each sample, this template requires an estimate of run precision for each gas. This is calculated using a minimum of 3 replicate values from the run (check standards) as the coefficient of variation, CV%) calculated as:

$$CV\% = \left| \frac{\sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}}{\mu} \right| \times 100\%$$

Additionally, the template requires each standard gases certified accuracy (as % on the gas certificate, as well as the check standard deviation for each gas).

B. NEON requires QA data to be submitted for each analysis batch using the template file batchQA_Dissolved Gas CSV file. This includes the observed concentration and known concentration values for each of the gas check standards run for a given batch.

C. NEON requires submission of long-term method and QA data (annually in January, or more often if there is a change in analytical standards or SOP). This information is submitted using the Lab Summary_Dissolved Gas CSV template. For each gas, it includes the method detection limit (MDL) calculated as the standard deviation times the t-value from a one-sided t-distribution at the 99% level. Use a minimum of seven replicates of a low-level check standard from across three or more runs to make this calculation. For 7 replicates, the t-value is 7-1 = 6 degrees of freedom = 3.14.

XIII. Troubleshooting
- A. Possible Problems
  - A. Unidentified peaks
    1. Peaks eluting at random retention times
  - B. Asymmetrical/multi peak/Fronting or Trailing peaks
  - C. No peaks
- B. Possible Solutions
  - A. Replace septum
  - B. Bake out column
  - C. Clean FID injector

XIV. Changing the septum
- A. All temperatures must be below 50 degrees
- B. Zero pressure in gas lines
- C. Unscrew injection port ring
- D. Carefully remove widget
- E. Pull out septum
- F. Put new septum in
- G. Replace widget
- H. Screw on metal ring
  - A. Tighten all the way
  - B. Back off ½ turn