CO_2-CH_4-N_2O by Gas Chromotography (Shimadzu) Version 1.1

Version Change Log:

Version	Date	Modifications
1.1	May 5, 2025	Details for QAQC steps added.

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 electrode, 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 compounds 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 its 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 chromatograph 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. 2 x 10mL
    L. Glass gas tight syringe: 1 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. 3 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 canister or cylinder to flush tubing
          ii. Attach nozzle on foil bag to tubing

iii. Open valve on foil bag by twisting left
iv. Fill until almost taught**; 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. Use a new 10mL syringe and needle for each **batch**
      i. 10mL syringe for N2
      ii. 10mL syringe for Mesa Standard
      iii. 1mL syringe for Levels 1-3 Mesa

D. Create 3 mid-level Check Standards for a batch of 40 samples

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	**0.2 (1mL)**	**19.8 (10mL)**	50	1	0.05
Level 2	MESA 0.025	**0.5 (1mL)**	**19.5(10mL)**	125	2.5	0.125
Level 3	MESA 0.05	**1(1mL)**	**19(10mL)**	250	5	0.25
Level 4	MESA 0.1	**2 (10mL)**	**18(10mL)**	500	10	0.5
Level 5	MESA 0.25	**5(10mL)**	**15(10mL)**	1250	25	1.25
Level 6	MESA 0.5	**10(10mL)**	**10(10mL)**	2500	50	2.5
Level 7	MESA 0.75	**15(10mL)**	**5(10mL)**	3750	75	3.75
Level 8	MESA 1.0	**20(10mL)**	------	5000	100	5

CHK STD 0.05	STD 0.05	**1(1mL)**	**19(10mL)**	250	5	0.25
CHK STD 0.1	STD 0.1	**2 (10mL)**	**18(10mL)**	500	10	0.5
CHK STD 0.25	STD 0.25	**5(10mL)**	**15(10mL)**	1250	25	1.25

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 20 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 septum needs to be changed
  - A. Septa need 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. Push 'Diag' button at top of instrument panel
      - a. Scroll to '3 Analysis Counter
      - b. Scroll to Septa counter
        - i. Press reset button on bottom of panel
      - c. Scroll to insert counter
        - i. Press reset button on bottom of panel
- 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. (1)Zero Air-60psi
      - b. (2)UHP Hydrogen-100psi
      - c. (3)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
        - i. Top Left: Inst Makeup: N2-5 psi
        - ii. Middle Top: Hydrogen-75 psi
        - iii. Middle Bottom: Air-50 psi
        - iv. Top Right:ECD Makeup: N2- 25psi

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 ID is number assigned by USUABL Lab
              b. Sample name is NEON barcode number
  D. All Standards and samples must be entered as "Unknown" in the Sample type column

E. 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. Right click on the "MethodFile" column and select 'Fill Down'

F. Run up to 40 samples per run

G. Run a check standard after every 20 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

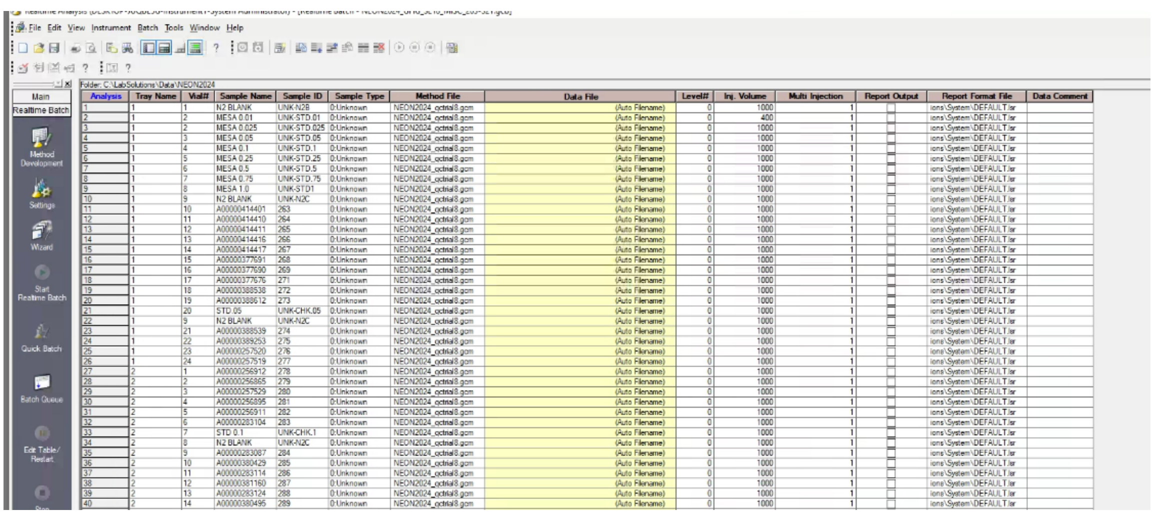


Figure 1. Example of Batch file sequence

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.

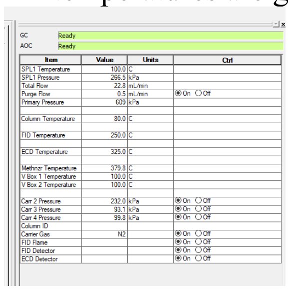
C. Samples are injected at 1 mL unless previously run and concentrations exceed the calibration limits
    A. If sample concentration is too high, inject a smaller volume according to level reported

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



GC	Ready
AOC	Ready

Item	Value	Units	Ctrl
SPL1 Temperature	100.0	C	
SPL1 Pressure	266.5	kPa	
Total Flow	22.8	mL/min	
Purge Flow	0.5	mL/min	On Off
Primary Pressure	609	kPa	
Column Temperature	80.0	C	
FID Temperature	250.0	C	
ECD Temperature	325.0	C	
Methnzr Temperature	379.8	C	
V Box 1 Temperature	100.0	C	
V Box 2 Temperature	100.0	C	
Carr 2 Pressure	232.0	kPa	On Off
Carr 3 Pressure	93.1	kPa	On Off
Carr 4 Pressure	99.8	kPa	On Off
Column ID			
Carrier Gas	N2		On Off
FID Flame			On Off
FID Detector			On Off
ECD Detector			On Off

Figure 2. Instrument and Autosampler ready

    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.

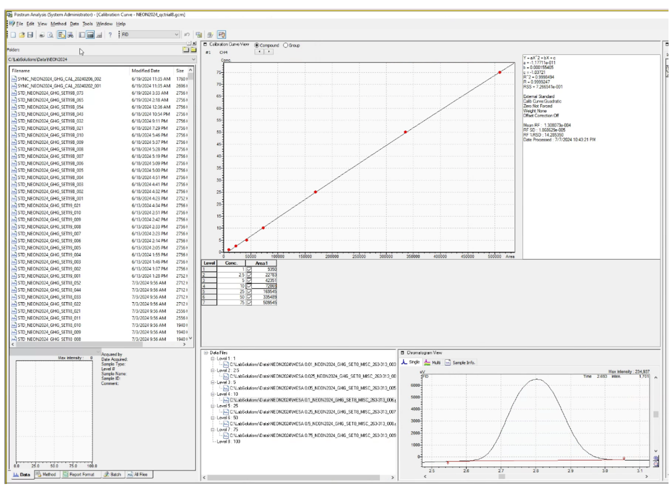


Figure 3. Example of Calibration Curve

    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"

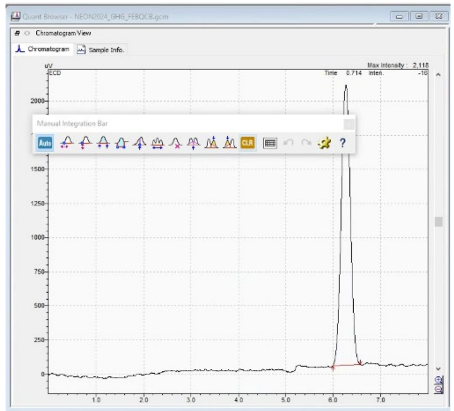


Figure 4. Example of good peak integration

4. Analytes with concentrations above the calibration range will need to be reanalyzed at a smaller volume according to the concentration reported
    a. i.e. if the methane is 150ppm (highest calibration level is 100ppmv), the injection volume should be halved to ensure 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

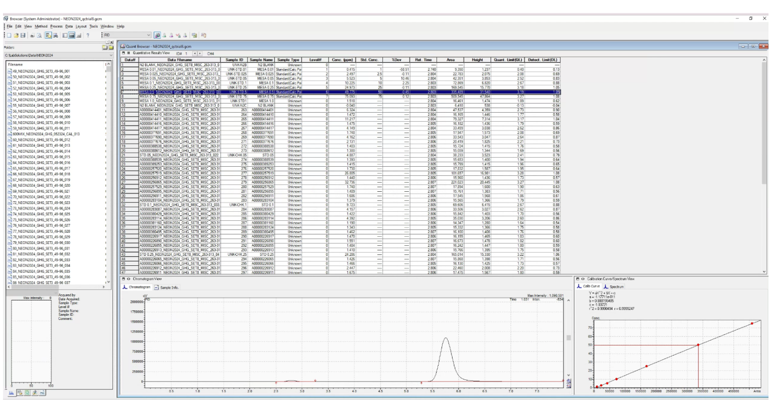


Figure 5. Example of QuantBrowser Data Report

XII. CREATING USUABL RAW DATA FILE

- A. Copy and paste Information for each analyte into Excel SDG Raw template (USUABL BOX file: NEON_DATA_"SDG_RAW")
  - A. Right click on top corner of Browser table "Data#"
    1. Select "copy entire table"
    2. Paste into blank excel SDG template "RAW" tab
    3. Toggle to each analyte (CH4, CO2 and N2O) and repeat
       - a. Drop down list at top of screen to toggle FID and ECD
- B. Raw data on first tab produced by instrument ("RAW") (Fig.5.)
  - A. All concentrations and QC information for all three analytes
  - B. Copy raw tab onto 2nd tab ("QC") and 3rd tab ("DATA")

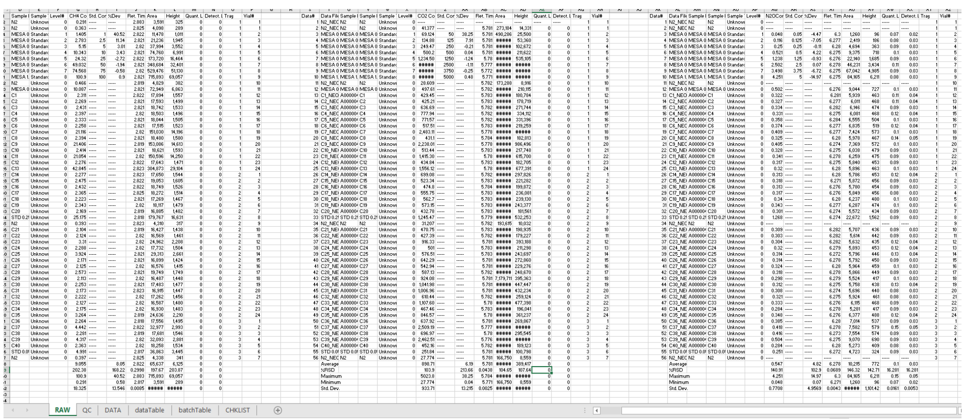


Figure 6. Raw Data Spreadsheet: Tab "RAW"

- C. QA/QC on second tab ("QC") produced by instrument (Fig.7) lab use only
  - A. Delete Sample data from this page leaving only QC
  - B. All concentrations and QC information for calibration and check standards and blanks only for each analyte
  - 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
    2. If the calculated RSD% is between 2-5%, check standards should be evaluated for any persistent problems with the rest of the run.
       - a. If Check Standards and Nitrogen blanks are within range, highlight cells in green.
         - i. Check standards RSD% below 5%
         - ii. Nitrogen blank concentrations below MDL
           1. CH4: 0.2 PPMV
           2. CO2: 40 PPMV
           3. N2O: 0.05PPMV

A	B	C	D	E	F	G	H	I	J	K	L	M
Data#	Data Filen	Sample ID	Sample Na	CH4 Conc.	Std. Conc.	%Dev	CO2 Conc.	Std. Conc.	%Dev	N2O Conc	Std. Conc.	%Dev
45	MESA 0.01	MESA 0.01	MESA 0.01	1.46	1	46	66.819	50	33.638	0.049	0.05	-2
46	MESA 0.02	MESA 0.02	MESA 0.02	2.745	2.5	9.8	129.533	125	3.6264	0.112	0.125	-10.4
47	MESA 0.05	MESA 0.05	MESA 0.05	5.435	5	8.7	260.644	250	4.2576	0.258	0.25	3.2
48	MESA 0.1_	MESA 0.1	MESA 0.1	10.131	10	1.31	498.784	500	-0.2432	0.513	0.5	2.6
49	MESA 0.25	MESA 0.25	MESA 0.25	24.36	25	-2.56	1,228.36	1250	-1.7312	1.241	1.25	-0.72
50	MESA 0.5_	MESA 0.5	MESA 0.5	48.752	50	-2.496	2,477.14	2500	-0.9144	2.502	2.5	0.08
51	MESA 0.75	MESA 0.75	MESA 0.75	74.652	75	-0.464	3,741.86	3750	-0.21707	3.687	3.75	-1.68
52	MESA 1.0_	MESA 1.0	MESA 1.0	100.964	100	0.964	5,021.86	5000	0.4372	5.03	5	0.6
54	MESA 0.1_	MESA 0.1	MESA 0.1	9.922	10	-0.78	490.925	500	-1.815	0.499	0.5	-0.2
21	STD 0.25_	STD 0.25	STD 0.25	24.993	25	-0.028	1,265.61	1250	1.2488	1.244	1.25	-0.48
43	STD 0.05_	STD 0.05	STD 0.05	5.054	5	1.08	251.291	250	0.5164	0.251	0.25	0.4
34	N2_NEON	N2	N2	0.089	-----	-----	18.996	-----	-----	-----	-----	-----
56	N2_NEON	N2	N2	0.018	-----	-----	19.971	-----	-----	-----	-----	-----

Figure 7. Quality Check spreadsheet: Tab "QC"

D. Sample Concentrations and Sample data on third tab ("DATA") (Fig.8)
   A. Report concentrations only in ppmv for all three analytes per sample
   B. Copy paste data from sample manifests to the right of the data, match sample ID with LabSample numbers and NEON barcodes.
      1. Double check all sample information is correct, highlight a minimum of 10% sample data that has been cross checked in green.
   C. Use Excel's "conditional formatting" function located on the home sheet to identify any duplicate barcodes, LabSample numbers or sample ID names.
   D. Copy/paste sample concentrations and information into 4th tab ("dataTable") (Fig.9)

Data#	Data Filen	Sample ID	Sample Name	CH4 Conc.	CO2 Conc.	N2O BCon	NEON2025_GHG_041725
42	B922_NEC	A00000467598	B922	2.436	525.983	0.335	
41	B923_NEC	A00000467581	B923	4.187	837.326	0.343	
40	B924_NEC	A00000467505	B924	2.998	481.337	0.34	
39	B925_NEC	A00000467504	B925	3.118	589.443	0.341	
38	B926_NEC	A00000467448	B926	2.583	789.344	0.363	
37	B927_NEC	A00000467449	B927	3.917	771.463	0.599	
36	B928_NEC	A00000467463	B928	2.693	500.815	0.362	
35	B929_NEC	A00000467480	B929	7.59	695.823	0.394	
34	B930_NEC	A00000467704	B930	2.785	486.227	0.343	
33	B931_NEC	A00000467705	B931	8.721	489.593	0.292	
32	B932_NEC	A00000467961	B932	2.801	457.187	0.333	
31	B933_NEC	A00000467960	B933	2.459	450.994	0.346	
30	B934_NEC	A00000467963	B934	2.706	452.108	0.363	
29	B935_NEC	A00000467962	B935	3.308	729.206	0.373	
28	B936_NEC	A00000467959	B936	3.076	737.353	0.512	
27	B937_NEC	A00000467964	B937	3.265	725.777	0.368	
26	B938_NEC	A00000469084	B938	2.862	464.734	0.347	
25	B939_NEC	A00000469085	B939	2.622	523.251	0.349	
24	B940_NEC	A00000467958	B940	2.515	458.933	0.336	
23	B941_NEC	A00000469087	B941	3.139	462.145	0.338	
20	B942_NEC	A00000469088	B942	2.441	470.205	0.339	
19	B943_NEC	A00000469086	B943	2.986	572.18	0.372	
18	B944_NEC	A00000467957	B944	3.076	564.881	0.37	
17	B945_NEC	A00000467956	B945	2.619	548.4	0.556	
16	B946_NEC	A00000449945	B946	2.816	758.405	0.364	
15	B947_NEC	A00000449944	B947	8.003	1,742.99	0.446	
14	B948_NEC	A00000468663	B948	2.368	551.164	0.366	
13	B949_NEC	A00000468664	B949	2.823	563.745	0.355	
12	B950_NEC	A00000468662	B950	2.525	607.019	0.359	
11	B951_NEC	A00000468711	B951	2.628	1,426.77	0.412	
10	B952_NEC	A00000468950	B952	2.34	821.916	0.358	
9	B953_NEC	A00000468949	B953	9.617	1,262.46	0.375	
8	B954_NEC	A00000468937	B954	2.963	560.935	0.418	
7	B955_NEC	A00000468936	B955	2.758	1,152.30	0.503	
6	B956_NEC	A00000467451	B956	2.626	785.964	0.337	
5	B957_NEC	A00000467452	B957	2.691	796.388	0.334	
4	B958_NEC	A00000467450	B958	3.087	1,041.79	0.313	
3	B959_NEC	A00000467464	B959	17.41	917.335	0.495	
2	B960_NEC	A00000467465	B960	17.071	890.651	0.468	
1	B961_NEC	A00000467453	B961	19.07	885.483	0.479	

Data#	domainID	dateShipped	shipmentI	senderID	sentTo	shipmentS	shipmentM	trackingN	quarantin	sampleID	sampleCode	sampleCla	namedloc	collectdate	
42	D07	2/27/2025	D072025	concklin@	Aquatic Bi	FedEx	Ground	# 7723741	N	WALK.ss.20250217.air	A00000467598	sdg_field	WALK	2E+07	B922
41	D07	2/27/2025	D072025	concklin@	Aquatic Bi	FedEx	Ground	# 7723741	N	WALK.ss.20250217.wat	A00000467581	sdg_field	WALK	2E+07	B923
40	D07	2/27/2025	D072025	concklin@	Aquatic Bi	FedEx	Ground	# 7723741	N	WALK.ss.20250224.air	A00000467505	sdg_field	WALK	2E+07	B924
39	D07	2/27/2025	D072025	concklin@	Aquatic Bi	FedEx	Ground	# 7723741	N	WALK.ss.20250224.wat	A00000467504	sdg_field	WALK	2E+07	B925
38	D05	3/4/2025	D052025	schoofs@	Aquatic Bi	FedEx	Ground	# 772453	N	CRAM.c0.20250210.air	A00000467448	sdg_field	CRAM	2E+07	B926
37	D05	3/4/2025	D052025	schoofs@	Aquatic Bi	FedEx	Ground	# 772453	N	CRAM.c0.20250210.wat	A00000467449	sdg_field	CRAM	2E+07	B927
36	D05	3/4/2025	D052025	schoofs@	Aquatic Bi	FedEx	Ground	# 772453	N	LIRO.c0.20250203.air	A00000467463	sdg_field	LIRO	2E+07	B928
35	D05	3/4/2025	D052025	schoofs@	Aquatic Bi	FedEx	Ground	# 772453	N	LIRO.c0.20250203.wat	A00000467480	sdg_field	LIRO	2E+07	B929
34	D09	3/18/2025	D092025	halljr@ba	Aquatic Bi	UPS	Ground	# 1ZWX13	N	PRLA.c0.20250317.air	A00000467704	sdg_field	PRLA	2E+07	B930
33	D09	3/18/2025	D092025	halljr@ba	Aquatic Bi	UPS	Ground	# 1ZWX13	N	PRLA.c0.20250317.wat	A00000467705	sdg_field	PRLA	2E+07	B931
32	D16	3/24/2025	D162025	palko@ba	Aquatic Bi	FedEx	Ground	# 880021	N	MART.ss.20250318.air.2	A00000467961	sdg_field	MART	2E+07	B932
31	D16	3/24/2025	D162025	palko@ba	Aquatic Bi	FedEx	Ground	# 880021	N	MART.ss.20250318.air.3	A00000467960	sdg_field	MART	2E+07	B933
30	D16	3/24/2025	D162025	palko@ba	Aquatic Bi	FedEx	Ground	# 880021	N	MART.ss.20250318.air	A00000467963	sdg_field	MART	2E+07	B934
29	D16	3/24/2025	D162025	palko@ba	Aquatic Bi	FedEx	Ground	# 880021	N	MART.ss.20250318.wat.2	A00000467962	sdg_field	MART	2E+07	B935
28	D16	3/24/2025	D162025	palko@ba	Aquatic Bi	FedEx	Ground	# 880021	N	MART.ss.20250318.wat.3	A00000467959	sdg_field	MART	2E+07	B936
27	D16	3/24/2025	D162025	palko@ba	Aquatic Bi	FedEx	Ground	# 880021	N	MART.ss.20250318.wat	A00000467964	sdg_field	MART	2E+07	B937
26	D16	3/24/2025	D162025	palko@ba	Aquatic Bi	FedEx	Ground	# 880021	N	MCRA.ss.20250304.air	A00000469084	sdg_field	MCRA	2E+07	B938
25	D16	3/24/2025	D162025	palko@ba	Aquatic Bi	FedEx	Ground	# 880021	N	MCRA.ss.20250304.wat	A00000469085	sdg_field	MCRA	2E+07	B939
24	D16	3/24/2025	D162025	palko@ba	Aquatic Bi	FedEx	Ground	# 880021	N	MCRA.ss.20250311.air.2	A00000467958	sdg_field	MCRA	2E+07	B940
23	D16	3/24/2025	D162025	palko@ba	Aquatic Bi	FedEx	Ground	# 880021	N	MCRA.ss.20250311.air.3	A00000469087	sdg_field	MCRA	2E+07	B941
20	D16	3/24/2025	D162025	palko@ba	Aquatic Bi	FedEx	Ground	# 880021	N	MCRA.ss.20250311.air	A00000469088	sdg_field	MCRA	2E+07	B942
19	D16	3/24/2025	D162025	palko@ba	Aquatic Bi	FedEx	Ground	# 880021	N	MCRA.ss.20250311.wat.2	A00000469086	sdg_field	MCRA	2E+07	B943
18	D16	3/24/2025	D162025	palko@ba	Aquatic Bi	FedEx	Ground	# 880021	N	MCRA.ss.20250311.wat.3	A00000467957	sdg_field	MCRA	2E+07	B944
17	D16	3/24/2025	D162025	palko@ba	Aquatic Bi	FedEx	Ground	# 880021	N	MCRA.ss.20250311.wat	A00000467956	sdg_field	MCRA	2E+07	B945
16	D18	3/26/2025	D182025	trauger@	Aquatic Bi	FedEx	Second da	# 880090	N	CARI.ss.20250304.air	A00000449945	sdg_field	CARI	2E+07	B946
15	D18	3/26/2025	D182025	trauger@	Aquatic Bi	FedEx	Second da	# 880090	N	CARI.ss.20250304.wat	A00000449944	sdg_field	CARI	2E+07	B947
14	D18	3/26/2025	D182025	trauger@	Aquatic Bi	FedEx	Second da	# 880090	N	TOOK.c0.20250310.air.2	A00000468663	sdg_field	TOOK	2E+07	B948
13	D18	3/26/2025	D182025	trauger@	Aquatic Bi	FedEx	Second da	# 880090	N	TOOK.c0.20250310.air.3	A00000468664	sdg_field	TOOK	2E+07	B949
12	D18	3/26/2025	D182025	trauger@	Aquatic Bi	FedEx	Second da	# 880090	N	TOOK.c0.20250310.air	A00000468662	sdg_field	TOOK	2E+07	B950
11	D18	3/26/2025	D182025	trauger@	Aquatic Bi	FedEx	Second da	# 880090	N	TOOK.c0.20250310.wat.2	A00000468711	sdg_field	TOOK	2E+07	B951
10	D17	3/26/2025	D172025	wardm1@	Aquatic Bi	UPS	Ground	# 1Z57R38	N	BIGC.ss.20250310.air	A00000468950	sdg_field	BIGC	2E+07	B952
9	D17	3/26/2025	D172025	wardm1@	Aquatic Bi	UPS	Ground	# 1Z57R38	N	BIGC.ss.20250310.wat	A00000468949	sdg_field	BIGC	2E+07	B953
8	D17	3/26/2025	D172025	wardm1@	Aquatic Bi	UPS	Ground	# 1Z57R38	N	TECR.ss.20250304.air	A00000468937	sdg_field	TECR	2E+07	B954
7	D17	3/26/2025	D172025	wardm1@	Aquatic Bi	UPS	Ground	# 1Z57R38	N	TECR.ss.20250304.wat	A00000468936	sdg_field	TECR	2E+07	B955
6	D05	3/27/2025	D052025	schoofs@	Aquatic Bi	FedEx	Ground	# 880090	N	CRAM.c0.20250310.air.2	A00000467451	sdg_field	CRAM	2E+07	B956
5	D05	3/27/2025	D052025	schoofs@	Aquatic Bi	FedEx	Ground	# 880090	N	CRAM.c0.20250310.air.3	A00000467452	sdg_field	CRAM	2E+07	B957
4	D05	3/27/2025	D052025	schoofs@	Aquatic Bi	FedEx	Ground	# 880090	N	CRAM.c0.20250310.air	A00000467450	sdg_field	CRAM	2E+07	B958
3	D05	3/27/2025	D052025	schoofs@	Aquatic Bi	FedEx	Ground	# 880090	N	CRAM.c0.20250310.wat.2	A00000467464	sdg_field	CRAM	2E+07	B959
2	D05	3/27/2025	D052025	schoofs@	Aquatic Bi	FedEx	Ground	# 880090	N	CRAM.c0.20250310.wat.3	A00000467465	sdg_field	CRAM	2E+07	B960
1	D05	3/27/2025	D052025	schoofs@	Aquatic Bi	FedEx	Ground	# 880090	N	CRAM.c0.20250310.wat	A00000467453	sdg_field	CRAM	2E+07	B961

Figure 8. Raw data spreadsheet: Tab "DATA"

Analysis D	Vial	Lab Sampl	File Name	runID	concentra	concentra	concentra	Sample Co	Sample ID	Sample Co	Lab Proce	Volume A	Remarks	Precision	Precision	Precision	CH4Certifi	CO2Certif	N2OCertif	CH4Check	CO2Check	N2OCheck	StandardPercentDev		
20250415		B922	B922_NEON2025_GHG_04	NEON2025	2.436	525.983	0.335	A0000046	WALK.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			
20250415		B923	B923_NEON2025_GHG_04	NEON2025	4.187	837.326	0.343	A0000046	WALK.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			
20250415		B924	B924_NEON2025_GHG_04	NEON2025	2.998	481.337	0.34	A0000046	WALK.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			
20250415		B925	B925_NEON2025_GHG_04	NEON2025	3.118	589.443	0.341	A0000046	WALK.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			
20250415		B926	B926_NEON2025_GHG_04	NEON2025	2.583	789.344	0.363	A0000046	CRAM.c0.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			
20250415		B927	B927_NEON2025_GHG_04	NEON2025	3.917	771.463	0.599	A0000046	CRAM.c0.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			20250415
20250415		B928	B928_NEON2025_GHG_04	NEON2025	2.693	500.815	0.362	A0000046	LIRO.c0.20	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			1.1
20250415		B929	B929_NEON2025_GHG_04	NEON2025	7.59	695.823	0.394	A0000046	LIRO.c0.20	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			0.5
20250415		B930	B930_NEON2025_GHG_04	NEON2025	2.785	486.227	0.343	A0000046	PRLA.c0.20	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			0.4
20250415		B931	B931_NEON2025_GHG_04	NEON2025	8.721	489.593	0.292	A0000046	PRLA.c0.20	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			-0.8
20250415		B932	B932_NEON2025_GHG_04	NEON2025	2.801	457.187	0.333	A0000046	MART.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			-1.8
20250415		B933	B933_NEON2025_GHG_04	NEON2025	2.459	450.994	0.346	A0000046	MART.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			-0.2
20250415		B934	B934_NEON2025_GHG_04	NEON2025	2.706	452.108	0.363	A0000046	MART.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			
20250415		B935	B935_NEON2025_GHG_04	NEON2025	3.308	729.206	0.373	A0000046	MART.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			
20250415		B936	B936_NEON2025_GHG_04	NEON2025	3.076	737.353	0.512	A0000046	MART.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			
20250415		B937	B937_NEON2025_GHG_04	NEON2025	3.265	725.777	0.368	A0000046	MART.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			
20250415		B938	B938_NEON2025_GHG_04	NEON2025	2.862	464.734	0.347	A0000046	MCRA.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			
20250415		B939	B939_NEON2025_GHG_04	NEON2025	2.622	523.251	0.349	A0000046	MCRA.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			
20250415		B940	B940_NEON2025_GHG_04	NEON2025	2.515	458.933	0.336	A0000046	MCRA.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	1.1	0.5	0.4			
20250415		B941	B941_NEON2025_GHG_04	NEON2025	3.139	462.145	0.338	A0000046	MCRA.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B942	B942_NEON2025_GHG_04	NEON2025	2.441	470.205	0.339	A0000046	MCRA.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B943	B943_NEON2025_GHG_04	NEON2025	2.986	572.18	0.372	A0000046	MCRA.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B944	B944_NEON2025_GHG_04	NEON2025	3.076	564.881	0.37	A0000046	MCRA.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B945	B945_NEON2025_GHG_04	NEON2025	2.619	548.4	0.556	A0000046	MCRA.ss.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B946	B946_NEON2025_GHG_04	NEON2025	2.816	758.405	0.364	A0000044	CARI.ss.20	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B947	B947_NEON2025_GHG_04	NEON2025	8.003	1,742.99	0.446	A0000044	CARI.ss.20	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B948	B948_NEON2025_GHG_04	NEON2025	2.368	551.164	0.366	A0000046	TOOK.c0.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B949	B949_NEON2025_GHG_04	NEON2025	2.823	563.745	0.355	A0000046	TOOK.c0.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B950	B950_NEON2025_GHG_04	NEON2025	2.525	607.019	0.359	A0000046	TOOK.c0.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B951	B951_NEON2025_GHG_04	NEON2025	2.628	1,426.77	0.412	A0000046	TOOK.c0.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B952	B952_NEON2025_GHG_04	NEON2025	2.34	821.916	0.358	A0000046	BIGC.ss.20	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B953	B953_NEON2025_GHG_04	NEON2025	9.617	1,262.46	0.375	A0000046	BIGC.ss.20	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B954	B954_NEON2025_GHG_04	NEON2025	2.963	560.935	0.418	A0000046	TECR.ss.20	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B955	B955_NEON2025_GHG_04	NEON2025	2.758	1,152.30	0.503	A0000046	TECR.ss.20	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B956	B956_NEON2025_GHG_04	NEON2025	2.626	785.964	0.337	A0000046	CRAM.c0.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B957	B957_NEON2025_GHG_04	NEON2025	2.691	796.388	0.334	A0000046	CRAM.c0.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			
20250415		B958	B958_NEON2025_GHG_04	NEON2025	3.087	1,041.79	0.313	A0000046	CRAM.c0.2	ok	L.Ward	1	N2O:NEOI	1.7	1.3	1.9	2	2	2	-0.8	-1.8	-0.2			

Fig.9 Raw data spreadsheet: Tab "dataTable"

E. Copy/paste check standard information into 5th tab (batchTable) (Fig. 10)
   A. %RSD is calculated in batchTable tab
   B. Copy/paste %RSD values only onto the dataTable tab and update %RSD (Fig. 7-right side of spreadsheet)
      1. There should be 2 sets for each analyte or 1 check standard per 20 samples. Update samples accordingly
         a. ie: 1st 20 samples use the 1st set, the 2nd 20 samples use the 2nd set, etc

analysisDa	qaReferer	runID	instrumer	analyte	standardT	qa_qc_ID	standardC	standardK	analyteUn	analyticalI	analyteQF	remarks	analyzedE	reviewedBy		
20250415	MESA_0.0	NEON2025	SHIMADZU	METHANE	CHECK ST/	STD 0.05	5.054	5	PPMV	1	0	N2O:NEOI	L.WARD		1.1	
20250415	MESA_0.0	NEON2025	SHIMADZU	CARBON D	CHECK ST/	STD 0.05	251.291	250	PPMV	1	0	N2O:NEOI	L.WARD		0.5	
20250415	MESA_0.0	NEON2025	SHIMADZU	NITROUS (	CHECK ST/	STD 0.05	0.251	0.25	PPMV	1	0	N2O:NEOI	L.WARD		0.4	
20250415	MESA_0.1	NEON2025	SHIMADZU	METHANE	CHECK ST/	STD 0.1	9.922	10	PPMV	1	0	N2O:NEOI	L.WARD		-0.8	
20250415	MESA_0.1	NEON2025	SHIMADZU	CARBON D	CHECK ST/	STD 0.1	490.925	500	PPMV	1	0	N2O:NEOI	L.WARD		-1.8	
20250415	MESA_0.1	NEON2025	SHIMADZU	NITROUS (	CHECK ST/	STD 0.1	0.499	0.5	PPMV	1	0	N2O:NEOI	L.WARD		-0.2	

Figure 10. Raw data spreadsheet: Tab "batchTable"

F. Go to 6th Tab ("QACHK") (Fig.11) and initial check boxes
   A. Quality Assurance requires 2 analysts initials before submitting to NEON
   B. 10% sample to ID match
      1. Check "Data" tab, highlight a minimum of 10% that have been checked and passed
      2. Run "conditional formatting" to find duplicates for SampleID, Sample Barcodes and LabID.
      3. Review check standards are highlighted in green
         a. Also review that the %RSD matches for dataTable and batchTable tabs
      4. Check that nitrogen blank samples are below the method detection limits
         a. $CH4$: 0.2 ppmv
         b. $CO2$: 40 ppmv
         c. $N2O$: 0.05 ppmv

	INITIAL 1	INITIAL 2
10% SAMPLE to ID MATCH	LJW	MAB
DUPLICATE CHECK (none)	LJW	MAB
CHECK STANDARD (<5%)	LJW	MAB
BLANK CHECK (<MDL)	LJW	MAB

	MDL (PPMV)
CH4	0.2
CO2	40
N2O	0.05

Figure 11. Raw data spreadsheet: Tab "QACHK"

XIII. 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 gas' 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.

XIV. 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

XV. 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