**Post GC Analysis Protocol**

By Mark E. De Guzman

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*Note*

JiJY or Jess does batch processing after the samples are processed through the GC. This stacks the data together into a report. Batch processed will contain a folder ‘BatchDB’ in the following data path: C:\IonVantage Projects original. The document contained within will have both raw and processed data with embedded calculations. Copy the cells over to a new excel sheet as values to process the data.

**Raw data extraction**

The batch processed file will contain three tabs: ‘Params,’ ‘F1CO2-report,’ & ‘F1CO2-log.’ Choose the one with ‘-log’ ending.

Copy the following columns:

|  |  |
| --- | --- |
| C | DataFileName |
| DP | RetTimeSecs (purple heading) |
| DR | MajorHeightnA (purple heading) |
| DS | TotalPeakArea1 (purple heading) |
| FC | DisplayDelta1 |

\*For quality check make sure that column ‘I’ in tab ‘-report’ matches with column ‘FC’ in the tab ‘-log.’

**Chromatograph visualization**

The program IonVantage is used to visualize the chromatograph. In the left portion of the user interface main display, this can be accessed by clicking the icon ‘View’ and then below it ‘Data Display.’

Open file through the icon ‘Open data file’ within the ‘Data display’ window and locate the folder that contains your data in C:\IonVantage Projects original\ (Format: YYYYMMDD\_GC\_name.PRO)\Data.

The middle and bottom panels are informative, but not necessary to visualize the peaks. They can be closed by clicking on the box and choosing the ‘Remove Graph’ icon. (box with red x). Right click on the orange legend 44:1 and click ‘Make only this visible’ to display the correct peak.

Once the data is visualized, the image can be zoomed by clicking and dragging to create a zoom box on the image. The icons with magnifying glass and cross double arrows can be used to undo zooming.

The image can be extracted as a .png by using the program Snipping Tool, which is accessed via the Window Start Up. The snipping tool is needed to print the image in black and white for better visualization.

**Peak identification**

For systemic chromatograph peak identification, process the standards first in the raw data extracted from earlier:

FAME

A representative sample

This will aid in naming the peaks for all the data in the spreadsheet as you can later rearrange the RefTimeSecs to name all your peaks. Coloring the FAME and named standards will help with locating these named peaks later.

± 2-3 seconds is the range used to encompass retention times. You can use the visualized data to double check.

Approximately 25 peaks will be named in every batch, but this can vary depending on the samples. Refer to the peak names table to identify specific peaks.

*Note*: the relative distance of the separation between peaks should be the same, what changes is the reference time so make sure you check for this for each batch.

*Quality check*

Pivot table at end after peak naming. Row = data file, Column label = peak name, value = count peak area. Make sure that 16:O, 13:O, & 19:O are in each sample, and for each fatty acid to make sure no 2s (or higher numbers) as this indicates double naming. If a given fatty acid is missing from many samples, check the data file and/or the visualized chromatogram to make sure that the peak doesn’t exist and not that it didn’t get named.

**Quantification**

After the quality check and naming are done for each individual batch, combine them and make another pivot table with all the samples together Row = data file, Column label = peak name, value = sum peak area.

13:O standard is used to tell the peak area k-factor for converting peak areas to ng of fatty acid.

Convert the peak area to nmol for count of fatty acids.

Jess has a template for calculations.

**FAQ**

Naming protocol. Why #:O?

# - indicates the number of carbon in the fatty acid chain

O – indicates the number of kinks (double bonds) in the chain

e.g. 16:1 ω7c

16 carbons, 1 double bond, small greek letter omega denotes presence of double bond, 7 indicates the position relative to the hydrophobic end, c refers to cis isomer (or use t for trans).