

SP045.3 Operation of the GC

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Gas Chromatography of Glycolipids

Materials:

Hexanes

Chloroform

Alditol Acetate Derivatives

Autoinjector vials (4 ml, Shimadzu)

Autosampler Vials (2 ml) with glass inserts and PTFE split caps (Waters)

Glass capillary pipettor and pipettes, 100 μ l

Pasteur pipets

Gas Chromatograph (GC-2014, Shimadzu)

Hydrogen cylinder (Airgas)

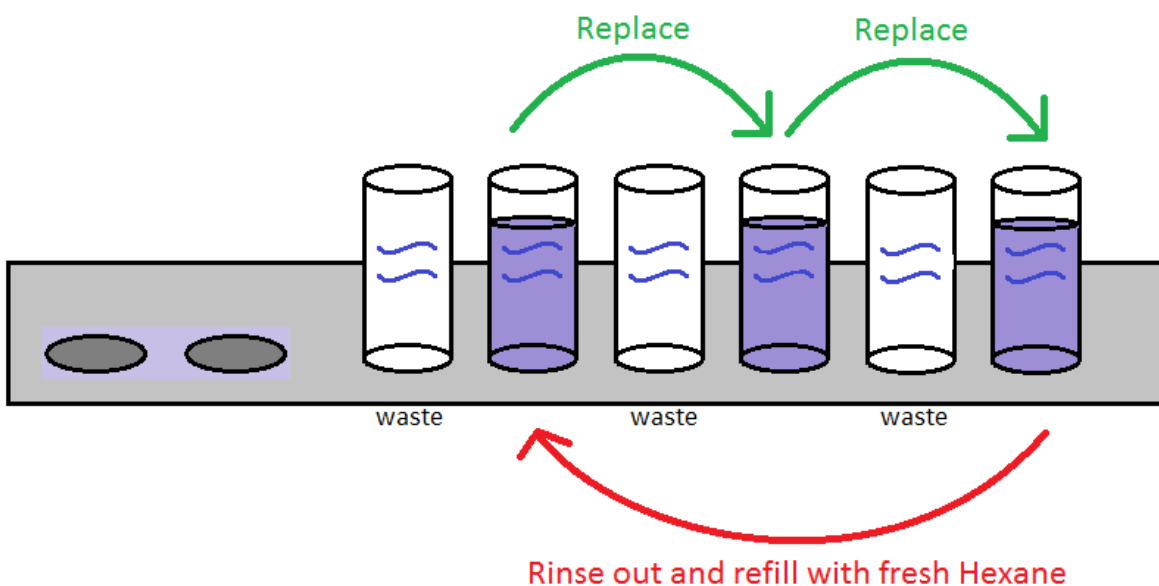
Nitrogen cylinder

Breathing air cylinder

Helium cylinder

Methods:

1. Re-suspend your standards and samples in 200 μ l chloroform and transfer into autosampler vials (2 ml) with small glass inserts. Also fill a separate 2 ml vial with chloroform, no insert, to be used as a blank.
2. Turn on all 4 gases connected to GC by turning counterclockwise at least one full rotation.
3. Before powering on the GC, change the hexane rinses out. The carriage in the Auto Injector tower slides out to the right. Fill each vial with at least 3 ml hexanes, working in a hood. Be careful not to invert the septum. The other vials are for rinse waste disposal, and do not need septa.



Make sure you push the rack back into the autoinjector all the way or else it will give you an error message.

4. Power on the GC by flipping the power switch on the right side towards the front.
5. Turn on the computer and log in.
6. Open up the Lab Solutions icon. The user name is stated as "admin" and there is no password.
7. Double click on the GC-2014 icon, the only one within the Instruments menu. You will hear a beep as the computer connects with the GC.
8. Under the Acquisition menu on the left side, click the System On button. Click Okay to download the instrument parameters.
9. You will hear the oven turn on. Hit the purple *MONIT* button on the GC instrument to view the main screen. The SPL injector will heat to 250°C, the FID detector will heat to 310°C, and the oven will warm to 60°C. This takes 5-10 minutes (note 1).
10. Once the temperature status light on the top of the GC screen is green, you may proceed with ignition. Push the *DET* button on the GC, then *PF1* to ignite the flame. Screen will say "ignition started", then "ignition finished" once flame is lit. This will take about 1 minute. Hit *MONIT* to return to the main screen. The picture of the flame under the FID symbol should be white, indicating that the flame is lit (note 2).
11. Now, the *TEMP* and *FLOW* lights should be green.
12. Put your standards and samples into the autosampler rack (carriage 1) and note their locations. Put the chloroform blank in position 1. Note that the place positions start from the robot arm and move towards the outer edge of the carriage.
13. On the computer go to Main submenu on the left side and click the Realtime batch icon. The previously used batch file will appear.
14. Make modifications to the position of each sample (Vial# column), as well as sample names. You can add rows for additional samples by right clicking where rows should be inserted, choosing Insert Row in the dropdown menu (note 3).
15. Click the File menu at the upper left side of the screen to save the overwritten batch, selecting your folder and naming the file itself. The data is defaulted to go directly to the file you specify.
16. Once the GC bar on the upper right side of the screen switches to "Ready," which will have a green background, click "Start Realtime Batch," under the Realtime Batch Menu on the left side of the screen.
17. The GC will now start your run; each vial takes about 50 minutes to complete (note 4).
18. At the end of the run, return to the Acquisition menu and click the "System Off" icon, then affirm that you want to turn it off. The temperatures will begin to drop as the oven is turned off.
19. You can also have the GC cool down automatically after a run, beneficial if you'd like to run a batch overnight (note 5).
20. Ensure that each gas has sufficient pressure for the next run. The gage on the right signifies the tank pressure, while that on the left is the regulator. Order a backup tank once the pressure drops below 1000 psi. With a pressure reading 200 psi or less, the run may be interrupted as all gases are necessary.

NOTES

1. You can also monitor the temperature on the right side of the computer screen.
2. Sometimes the ignition will take a few attempts before it says "Ignition finished." It is set to reignite automatically if it is not successful the first few times.
3. Also make sure the chloroform blank is used at the beginning and after the neutral sugar stands have been ran. This will show if there is any carryover of sample from the previous one. The blank may be ran after each sample in triplicate if preferred. A run of LAM, PIM6, and LM together will often access the blank 3 or 4 times to check for such carryover signal. Copy and paste the Sample Name column to the Sample ID column. Ensure that the Sample Type is "2:Control" for each NS standard by clicking within those cells and using the dropdown arrow. All others should be set to "0:Unknown." Within the Level# column, set the standards to "1," all others to "0." Click the "Report Out" square for any you would like to see a generic spectrum printed to D139. Usually this means 1 standard, and 1 from each group of triplicates. The final spectrum will be accessed from the PostRun software, however, so this is not crucial.
4. Stay and make sure the robot arm transfers the chloroform blank to the beveled vial holder within the Auto Injector carriage. Sometimes there is a carriage error which will prevent further sampling, and the instrument may need to be shut down and started again.
5. Start your batch program as above. After the machine completes the injection of the first sample (not the first blank or standard injection) you can go to "tools" on the top, then select "auto system off/on" then check "auto system off" and "after analysis". A box will pop up—DON'T CLOSE IT! Just move it off to the side and leave it there, closing it will cancel the auto cool down. You will still need to come in and power off the GC and gases as soon as you get in the next morning.