

Primary metabolism analysis by GC-TOF MS

Version: 1
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Summary: Description of standard setting for analysis of derivatized samples ready for injection into a Leco Pegasus III or Pegasus IV gas chromatograph-time of flight mass spectrometer.

Reagents and Materials:

Chemicals and consumables are described in the SOP “Sample preparation blood for GCTOF MS”.

Reagent/Material	Vendor	Stock Number
GC-TOF Pegasus III or IV instrument from LECO corp.		
Agilent 6890 GC/5975		

Protocol:

A General parameters for GC-TOF MS profiling for primary metabolism

Injection with Gerstel Cold Injection system and Agilent 7890A gas chromatograph:

- Method Name: “quick_Splitless_ms_alex”
- Injector: Gerstel MPS/CIS injector
- Injector Mode: Splitless
- Purge Time: 25 sec
- Purge Flow: 40mL/min
- Injector Vol: 0.5µL
- Carrier gas: Helium
- Carrier gas flow: 1ml/min
- CIS Temp Program:

	Temp	Rate	Equilibr.	Time
Initial	50°C		0.5min	0min
Final	275°C	12°C/sec		3min

- Gas chromatograph temperature program:

	Temp	Rate	Time
Initial	50°C		0.5min
Final	330°C	20°C/min	10min

- Gas chromatograph column:
RTX-5MS, 30m long, 0.25mm i.d. 0.25mm film duraguard with 10m guard to column.
(Restek order # 12723-127)

Mass spectrometry parameters with Leco Pegasus III or IV:

- Solvent Delay: 335 sec (varies with time, confirm with each column)
- Ion Source Temp: 250°C
- Scan Rate: 17specs/sec
- Detector Voltage: 1750 V to 1950V (settings according to age of detector)


B Maintenance for GC-TOF MS profiling for primary metabolism


Materials

- New Rtx5-Sil column
- Interface nut
- Injector nut
- Ferrules, grey and gold
- Methanol
- Ethyl acetate
- Kimwipes
- Column cutter/adaptor
- Interface pole
- “Dentist tool”
- Magnifying glass
- Wrenches, “G” and “T” ; Allen wrench
- MLE Toolkit (located in LECO cabinet)
- Red septa
- Flashlight (optional)
- Agilent ruler/cutter
- Sharpie
- Adapter heads
- Glass liners
- Push pin (tack)
- Black assembly tool
- Isopropanol
- Test tube brush, very small

B1 How to change a gas chromatography capillary column in GC-TOF (AgilentGC-Pegasus III) system

Method

1. Cool oven to 30°C
 - a. Press **Oven** on the front of the Agilent GC
 - b. Enter **30** on keypad
2. Cool inlet (CIS temp) to 20°C
 - a. From Maestro, click **Method**
 - b. Click **Load**
 - c. Select *20C for injector plate change and clean method*
 - d. Click **Get Ready**
3. Turn the electrical OFF by moving the switch, located on the front of the instrument, to the  position

4. Wait approximately 1.5 hours until the ion source temperature is $< 100^{\circ}\text{C}$
5. Close both the Maestro and ChromaTOF softwares
6. Turn the vacuum OFF by moving the switch, located on the front of the instrument, to the  position
7. Slowly vent the turbo pump by carefully twisting the vent knob until the “hissing” noise stops.
8. Close the vent knob.
9. Unscrew the pump cable (no pump cable for CORE)
10. Remove the safety pin from the table and carefully separate the table
11. Open the oven door

To install column at injector site:

12. Remove the old column from the injector end and insert new column in following the laboratory protocol *Cutting the Column*
 - a. NOTE: On the new column, “Guard Column End” goes to the injector. “Guard” always refers to injector end!
 - b. NOTE: If reusing the ferrule or adapter, sonicate in ethyl acetate for 5 minutes to clean

To install column at detector site:

13. Inside the oven, unscrew the round plate (facing out) to reveal the transfer line and old column (CORE GC does not have this cover, transfer line is in plain sight)
14. Unscrew the transfer line nut and carefully pull the old column out of the detector end
15. Break off the nut (by breaking the old column) and remove the grey ferrule from within
 - a. NOTE: Use a paperclip/tack to remove ferrule from the nut if it's stuck. If it can't be removed, use a new nut.
16. Unwind about 2 feet of the new column and insert the grey ferrule and nut
17. Cut about 10 cm of the new column and wipe clean with a Kimwipe and methanol
18. Feed the new column through the interface pole with the transfer nut lightly (finger tight) screwed on, leaving about 4-6mm sticking out the other end
19. Using a Sharpie, and keeping 4-6mm sticking out of the end of the interface pole, mark the column position on the opposite side containing the nut and ferrule
20. Remove the column from the interface pole and feed the column into the actual interface, screw in the nut and ferrule VERY TIGHTLY and make sure column is the correct length based on the Sharpie mark.
 - a. Ensure threading on nut is good, you should be able to screw in the nut almost all the way by hand, and tighten with wrench at the very end; otherwise, select a more appropriate nut.
21. Inside the oven, screw the round silver cover back into place (skip for CORE GC)
22. Carefully push back the table, replacing the safety pin; close oven door.
23. Screw the pump cable back on the turbo pump (skip for CORE GC)
24. Turn the vacuum ON (opposite of step 3)
25. Open both the Maestro and ChromaTOF software
26. Wait until the vacuum pressure reaches $1\text{E}-7$; takes at least 3 hours (pressure reading is located on the bottom-right corner of the LECO software)
27. Turn the electrical ON (opposite of step 6)
28. After transfer line reaches 280°C and the ion source reaches 250°C , let instrument sit for another hour (to reduce moisture in system) before conditioning column.
29. Condition the column twice using the following temperature program:

Initial temp: 50°C
Initial time: 1 minute
Rate 1: $10^{\circ}\text{C}/\text{minute}$
Final temp 1: 100°C
Final time 1: 10 minutes
Rate 2: $10^{\circ}\text{C}/\text{minute}$
Final temp 2: 330°C
Final time 2: 20 minutes

30. At this time, also adjust CIS temp to 250°C for at least 15 minutes before bringing it down to 50°C (change temperatures by loading liner condition or sample run method).
31. Perform a leak check by following the laboratory protocol performing a *Leak Check*.
32. Condition the column with corn samples and quality controls, following the laboratory protocol *conditioning the Column*.

B2 How to cut the Gas Chromatography capillary column after analysis of complex biological matrices to restore the system.

Method:

1. Cool oven to 30°C.
 - a. Press **Oven** on the front of the Agilent GC.
 - b. Using the keypad, type **30**, press **Enter**.
2. Cool the inlet to 20°C.
 - a. On Maestro software, click **Method** → **Load**.
 - b. Select *20C for injector plate change and clean method* and hit “▶ Get Ready” button.*
3. Turn gas saver OFF.
 - a. Press **Back Inlet** on front of GC.
 - b. Scroll down till Gas Saver, press OFF on keypad.
4. Open the oven door. Use wrench “T” to unscrew and remove the adapter nut.
5. Remove the column by pulling on the ferrule by hand. If successful, skip to step 9.
 - a. Note: If the ferrule is stuck, you will need to remove the back adapter (**steps 6-8**) and try to pry it off the adapter with pliers. If the ferrule becomes unusable, you will need to use a new ferrule and follow the guidelines in steps 9b, 12a, and 19a.
6. Insert the septa (minimize air exposure inside column) to the end of the column.
7. Remove the round metal plate covering adapter.
8. Use wrench “G” to unscrew the adapter, and replace with a new adapter.* Replace metal cover as well.
 - a. Do not over-tighten adapter, adapter should go on easily by hand, only use wrench at the very end to tighten (finger-tight).
9. Measure approximately 10 cm and cut the column with the Agilent ruler/cutter.
 - a. If ferrule is undamaged/relatively new, and still fits snugly around column, simply pull it down further along column before making the cut.
 - b. If ferrule is damaged/ unusable, replace with new ferrule before cutting column.*
10. Examine the new cut with the magnifying glass to ensure a clean, straight cut.
 - a. If inadequate, make another cut a few mm down.
11. Clean the tip of the column with MeOH on a kimwipe.
12. Using a ruler, measure the column sticking out from the front of the ferrule out to 1.7 cm.
 - a. If you have to use a new ferrule, it will be very loose. Tighten first by installing column back into injector, and then removing it to measure 1.7cm. Ferrule should snugly fit around column.*
13. Carefully thread column back up into adapter.
14. Firmly push the ferrule into place by hand.
15. Screw the adapter nut back into place.
16. Position the column so that no part of it is touching the sides of the oven—to avoid uneven heating of column.
17. Close the oven door.
18. Turn the gas saver back ON. Check if pressure holds.
19. Increase the oven temperature back to 50°C.
 - a. If new ferrule is installed, increase CIS temp to 250C (load Maestro liner condition method) for ten minutes, and bring it back down to 50C (load Maestro sample run method)

20. Perform a leak check by following the laboratory protocol *performing a Leak Check*. Make sure CIS temp is at 50C, and pressure is at 7.7psi before running leak check.


NOTES:

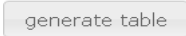

- Step 2b : Cools CIS temp to 20C so injector is not extremely hot while handling adapter and column.
- Step 8: If old adapter is still in good condition, clean old adapter using compressed air to blow out dirt or sonicate for 5 min in hexane at room temperature.
- Step 9b: Column ALWAYS needs to be cut every time it is inserted through ferrule. Graphite deposits from ferrule may end up around column opening and possibly inside column; this should be avoided.
- Step 12a: To tighten ferrule around column, insert the column with ferrule into injector, gently push ferrule onto adapter to fit, screw adapter nut back on, unscrew, pull out ferrule/column, and the ferrule should now be fitted snugly around column. Repeat if necessary and then proceed with measuring and inserting properly.
- Step 19a: heating CIS injector will help provide a better seal as metal parts expand and come together/tighten; especially helpful if leak check is not passing.

B3 How to clean and assemble liners for Gerstel CIS injector system**Method**

1. Remove glass liner from transport adapter (liner heads) and dispose used glass liners in the broken glass waste container
2. Unscrew smaller piece from larger head using an Allen wrench
3. Remove the septa from smaller piece using the push pin without scratching the surface of metal piece.
4. Rinse inside of adapter head with isopropanol, scrubbing through with the test tube brush. Take care not to squirt isopropanol directly on rubber O-rings (the rubber will dry out/become brittle over time).
5. Smaller piece is sonicated for 5 minutes in isopropanol.
6. Leave all pieces on Kimwipe to dry
7. When dry, insert a new septa and screw in smaller piece into the adapter head using an Allen wrench
8. Attach the adapter to a new glass liner using the black assembly tool
 - a. Insert a glass liner (baffled end first) into the assembly tool
 - b. Place the assembled adapter onto the assembly tool (septa facing up)
 - c. Press down on the top of the adapter a few times to firmly attach the liner to the adapter
 - d. Pull out the newly assembled liner and tug gently on glass liner to make sure it is snugly attached to adapter head.

C How to make a sequence on LECO ChromaTOF & Gerstel Maestro software**C1 Creating data files from a Minix study**

1. Sign in to MiniX (<http://minix.fiehnlab.ucdavis.edu/>)
2. Click on **list studies** and select study of interest (see LAB protocol for *Creating a MiniX study*)
3. On left side menu list, select  **create aquisition table**
4. Under **Binbase specific settings**,
 - a. select which instrument will be used: **core GC-TOF -a** or **research GC-TOF -b**.
 - b. check all boxes; these are for blanks and QC6s, and the QC6-QC1 curve.
5. Under **sample name generation settings**,
 - a. select date you will start, if unsure, estimate.
 - b. the date you enter will serve as the first six digits of your filenames for the first 50 samples; if you have more than 50, the next 50 will have the date for the following day.
6. Under **data acquisition settings**,

- a. for research instrument, select the only option available for all three methods.
 - b. for CORE instrument, there are no options available, so leave as is.
7. When finished, click  – it will display the acquisition table you have just created.
 8. Click on **view study**. Under **Attachments**, download the acquisition table onto GC-TOF computer by clicking  **95240-aquisitionTable.seq**

C2 Creating a ChromaTOF sequence table from miniX study

9. Transfer the downloaded acquisition file to **minix folder** located in **gctof** drive located on network jupiter.fiehnlab.ucdavis.edu
 - a. save file by date, followed by miniX ID and brief description; if it is a CORE study, for example: CORE_120628_MX95240_rafael plants
10. On ChromaTOF, in the **Database** window (top left screen), click on **Acquisition Tasks**
 - a. On menu bar (very top row), click **Acquisition Tasks** → **Sequence Table** → **Load...**
 - b. Select file of interest and open to load file onto Acquisition Tasks screen.
 - c.

Vial	Name	GC Method	MS Method	DP Method	Folder
1	120628bmsLiner condition	quick-splitless_working_PT 25 sec liner condition	MassSpec OFF liner condition	fiehn default	Acquired Samples
1	120628bmsLiner condition	quick-splitless_working_PT 25 sec liner condition	MassSpec OFF liner condition	fiehn default	Acquired Samples
1	120628bmsLiner condition	quick-splitless_working_PT 25 sec liner condition	MassSpec OFF liner condition	fiehn default	Acquired Samples
3	120628bmsbl	quick-splitless_working_PT 25 sec	quick_1850V_17spec per sec	fiehn default	Acquired Samples
4	100526bmsqc6	quick-splitless_working_PT 25 sec	quick_1850V_17spec per sec	fiehn default	Acquired Samples
5	100526bmsqc5	quick-splitless_working_PT 25 sec	quick_1850V_17spec per sec	fiehn default	Acquired Samples
6	100526bmsqc4	quick-splitless_working_PT 25 sec	quick_1850V_17spec per sec	fiehn default	Acquired Samples
7	100526bmsqc3	quick-splitless_working_PT 25 sec	quick_1850V_17spec per sec	fiehn default	Acquired Samples
8	100526bmsqc2	quick-splitless_working_PT 25 sec	quick_1850V_17spec per sec	fiehn default	Acquired Samples
9	100526bmsqc1	quick-splitless_working_PT 25 sec	quick_1850V_17spec per sec	fiehn default	Acquired Samples

11. The beginning of each sequence starts with three liner conditions, followed by a reagent blank and QC6, 5, 4, 3, 2, 1 in this order:
 - a. Leave all other columns blank. By default, *Type* and *Tray* columns are automatically filled. *Type* is always “Sample”, and *Tray* contains unique minix number for each sample. Leave *Tray* cells empty for liner conditions or any other non-minix samples.
 - b. To fill in columns, highlight column of interest, or cell, for example, right-click → **Fill** → *quick-splitless_working_PT 25 sec* → **OK**
 - c. Click on the “+” sign to add a row above selected row or the “-” to delete selected row(s). Multiple rows can be deleted, but rows can only be added manually
 - d. Leave vial 2 empty, and samples start at vial 10
 - e. Liner conditioning occurs every 10 samples
 - i. Insert 5 additional rows and copy and paste the *Tray*, *Vial*, and *Name* of the 3 liner conditions, the reagent blank and QC6.
 - f. End the entire study with a single QC6 run.

- g. Note that the tray only holds 98 vials. If you have more than this number of vials, you need to restart numbering at #10 after #98.
- 12. Re-save this newly modified sequence by overwriting the existing file in miniX folder:
Acquisition Tasks → Sequence Table → Save... CORE_120628_MX95240_rafael plants
- 13. Copy the entire column containing the vial numbers into an Excel spreadsheet (used to create the Maestro sequence).

Note: Creating a ChromaTOF sequence from a non-miniX study

- 1. Follow steps 12-14 from above using the “+” icon to add lines.

C3 Creating a Maestro sequence from ChromaTOF sequence

- 1. On Maestro menu bar click **Sequence → New...**
 - a. Or use an existing template, but first **Sequence → Save As** a new sequence file before proceeding.
 - b. Click on <icon> or **Sequence → Edit** to open up Maestro sequence table for editing.
- 2. On ChromaTOF, highlight entire **Vial** column, and copy **Ctrl+C**.
- 3. On Maestro, highlight first cell of **Vial** column (entire cell should be completely shaded blue), and paste **Ctrl+V**.
 - a. Do not worry about having the correct number of lines; Maestro will add lines as necessary.
 - b. Leave screen alone even if it looks like it is going to freeze after you paste; sometimes it does that with a brand new sequence. Just give software some time.
- 4. To add rows: select a row and click the **Insert** icon at the top of the screen; this inserts a new row above highlighted row.
- 5. Add a **liner change (NEWLINER)** before every liner conditioning set, and fill in all other necessary information as follows:

Vial	Method	Tray	Injector	Sample Name
1	NEWLINER	ALXTray1, VT981	ALXI1	
1	hot inj_liner condition.mth	Tray1, VT98	ALXI1	100526_Liner condition
1	hot inj_liner condition.mth	Tray1, VT98	ALXI1	100526_Liner condition
1	hot inj_liner condition.mth	Tray1, VT98	ALXI1	100526_Liner condition
2	QC-Cold-inject-50C(100606 yl).mth	Tray1, VT98	ALXI1	100526bserb
3	QC-Cold-inject-50C(100606 yl).mth	Tray1, VT98	ALXI1	100526bseqc06
4	QC-Cold-inject-50C(100606 yl).mth	Tray1, VT98	ALXI1	100526bseqc05
5	QC-Cold-inject-50C(100606 yl).mth	Tray1, VT98	ALXI1	100526bseqc04
6	QC-Cold-inject-50C(100606 yl).mth	Tray1, VT98	ALXI1	100526bseqc03
7	QC-Cold-inject-50C(100606 yl).mth	Tray1, VT98	ALXI1	100526bseqc02
8	QC-Cold-inject-50C(100606 yl).mth	Tray1, VT98	ALXI1	100526bseqc01

- 6. To check and confirm sequences on ChromaTOF and Maestro match:
 - a. Highlight the entire sequence table by clicking the top-left corner of the sequence table, and copy/paste to Excel spreadsheet.
 - b. Delete the newliner positions in the Maestro.
 - c. Confirm vial numbers in both columns (LECO and Maestro) match.
- 7. Click **OK** on the Maestro sequence table.
 - a. You will lose changes made if you close sequence table window without clicking **OK**.
- 8. Save the sequence: **Sequence → Save As...** and save under C:\Program files\Maestro\1\Sequence using the same name you saved sequence in ChromaTOF.

IMPORTANT: *In order to avoid cross-contaminations and artifact formation, disposable consumables are used (Eppendorf plastic tubes, plastic pipette tips)*

DISPOSAL OF WASTE: *Chemicals are disposed into appropriate bottles in the lab under the fume hood before monthly disposal collection. Glass vials and consumables are collected into the plastic bags and stored under the fume hood in lab before monthly disposal. Other GC-TOF waste (rubber seals, O-rings etc.) can be disposed into regular waste.*

References: Fiehn O, Wohlgemuth G, Scholz M, Kind T, Lee DY, Lu Y, Moon S, Nikolau BJ (2008) Quality control for plant metabolomics: Reporting MSI-compliant studies. *Plant J.* 53, 691-704