Lipidomic Analysis by UPLC-QTOF MS

Version: 1
Edited by: Oliver Fiehn

Summary: Lipidomic analysis by UPLC-QTOF mass spectrometry

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Agilent 1290 UPLC-6530-QTOF		
Pipettes calibrated following SOP006_2003		
Ultrasonicator		
Waters Acquity CSH C18 2.1x10 0mm 1.7 µm Column		
Waters Acquity VanGuard CSH C18 1.7 µm Pre-column		
Pipettes calibrated following SOP006_2003		
Agilent Tune Mix: G1969-85000		
Acetonitrile	J.T. Baker LC/MS Grade, 4 L	9829-03
Formic Acid	Fluka Mass Spec Grade	94318-250mL-F
Ammonium Formate	Fluka, Mass Spec Grade	70221-25G-F
Isopropanol	Fisher	A464-4
Agilent Capillary for 6530 (G1960-80060)		
Agilent 0.17ID (green) metal tubing: 90 cm 5065-9963 and 20 cm (5065-9931)		
Red Agilent Peek Tubing 5 meters (0.13 ID) (5042-6461)		
Plastic Agilent Connectors (for peek tubing) (0100-1516)		
Stainless Steel Agilent Fitting (5062-2418)		

Protocol:

3.1 Pre-run procedures

3.1.1 Instrument tuning (Instrument in Tune mode)

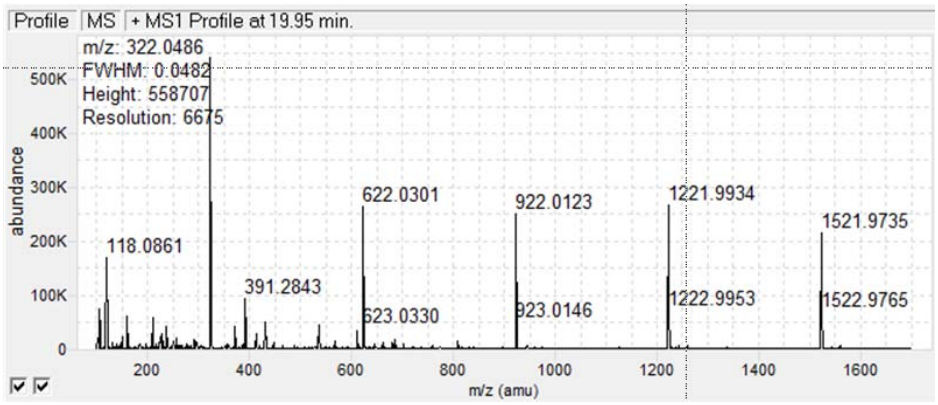
a. Use “Standard Tune” before each run of 300 sample batch.

b. Use the “Tuning Solution” (see preparation of solutions below) for the instrument tuning.

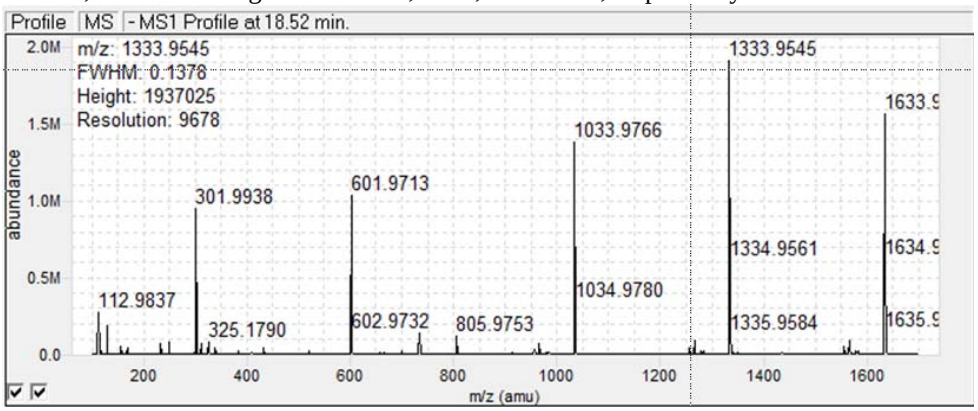
c. The mixture for the instrument tuning must be prepared fresh at the beginning of each 300 sample batch.

d. Print the tune report from the standard tune.

- In ESI(+), check the profile of the calibrant and the intensity of ions *m/z* 322.0481; *m/z* 622.0290; and *m/z* 922.0098, which must be higher than 400k, 200k, and 200k, respectively.



- In ESI(–), check the profile of the calibrant and the intensity of ions *m/z* 301.9981; *m/z* 601.9790; and *m/z* 1033.9881, which must higher then 800k, 800k, and 1100k, respectively.

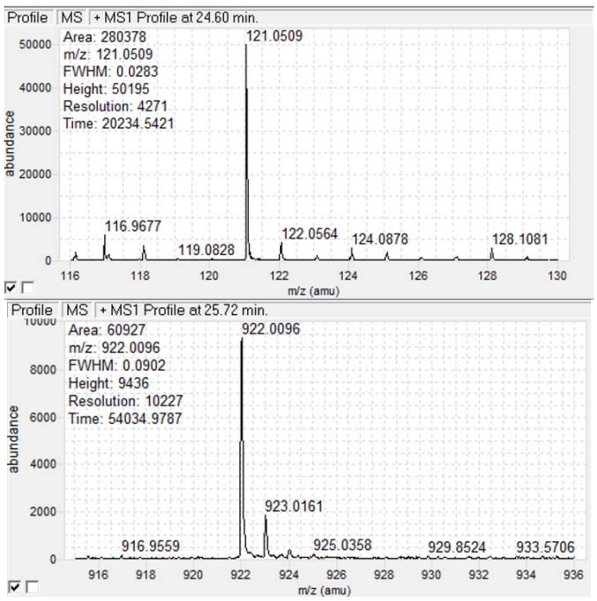


e. If the intensity of even one of the selected ion is below this value clean the ion source and repeat the instrument tuning.

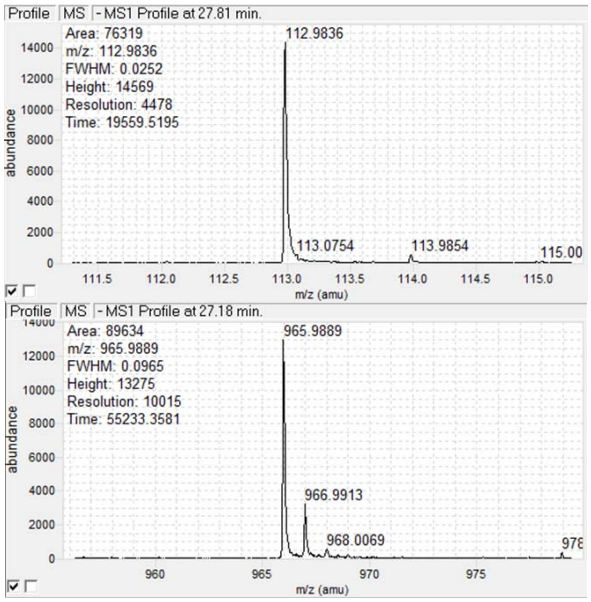
3.1.2 Check Reference ions (Instrument in Acquisition mode)

a. Use the "Reference Ion Mass Solution" (see preparation of solutions below) for mass correction during the analyses (lock mass).
b. The mixture for the reference ion solution must be prepared fresh at the beginning of each 300 sample batch.
c. The reference mass solution is pumped at a flow rate of 0.150 mL/min and split 1:10 prior entering the mass spectrometer.
d. Check the following reference ions:

- In ESI(+), check the intensity of ions *m/z* 121.0509 and *m/z* 922.0098, which should be between 40–60k and 8–12k, respectively. Adjust recipe and flow rates to attain this intensity.



- In ESI(–), check the intensity of ions *m/z* 112.9856 and *m/z* 966.0007, which should be between 10–20k and 10–20k, respectively. Adjust recipe and flow rates to attain this intensity.



3.2 New column installation

a. Purge the pumping system of any buffer-containing mobile phases and connect the inlet end of the column to the injector outlet.

b. Flush column with 100% acetonitrile by setting the pump flow rate to 0.1 mL/min and increase the flow rate to 0.6 mL/min over 5 min.

c. When the mobile phase is flowing freely from the column outlet, stop the flow and attach the column outlet to the ion source of the mass spectrometer.

d. Gradually increase the flow rate with 100% acetonitrile by setting the pump flow rate to 0.1 mL/min and increase the flow rate to 0.6 mL/min over 5 min.

e. Monitor the backpressure until a steady values is achieved.

f. Stop the flow and flush column with mobile phase (A) and (B) (see preparation of solutions below) at a ratio of 50:50 by setting the pump flow rate to 0.1 mL/min and increase the flow rate to 0.6 mL/min over 5 min.
g. Monitor the backpressure until a steady values is achieved. For a new column a value of backpressure should be 500–550 bar at the beginning of the injection (elution at 40% of the mobile phase (B), see preparation of solutions below).
h. Inject 5 blank (methanol). Record the lowest and highest value of backpressure for the first and the last sample injected.
f. Inject 10 citrate plasma samples. Record the lowest and highest value of backpressure for the first and the last sample injected.
NOTE: Use a new column after 300 sample injections. The UPLC column must be coupled to a VanGuard pre-column. The VanGuard pre-column is replaced after 150 sample injections. The number of injections (both solvents and plasma samples) is recorded by an operator in Excel file stored at <D>:\\<MassHunter\Methods\TEDDY methods>\TEDDY_Injections.XLS.

3.3 Preparation of solutions
a. Preparation of Tuning Solution
- 88.5 mL acetonitrile
- 1.5 mL H_2O
- 10 mL Agilent Low Concentration ESI Tuning Mix
- 5 µL 322 Reference Ion (sonicate before use)
- Degas by sonication for 5 min
- 100 mL will typically last months

b. Preparation of Reference Mass Solution
- 95 mL acetonitrile
- 5 mL H_2O
- 100 µL 5 mM 921 Reference Ion (sonicate before use)
- 125 µL 5 mM TFA Reference Ion (sonicate before use)
- 125 µL 10 mM Purine Reference Ion (sonicate before use)
- Degas by sonication for 5 min

c. Mobile phase A (60:40 ACN:water + 10 mM Ammonium Formate + 0.1% Formic Acid)
1. Pre-rinse three times 1 L glass bottle with pure acetonitrile
2. Measure exactly 600 mL of acetonitrile in a pre-rinsed graduated cylinder and add them to the 1 L glass bottle.
3. Measure exactly 400 mL of MilliQ water in a pre-rinsed graduated cylinder and add them to the 1 L glass bottle.
4. Add 1 mL formic acid
5. Weight 0.630 g of ammonium formate and add them to the glass bottle
6. Sonicate for 10 min at room temperature until all the ammonium formate is dissolved.
7. 1 L will last for around 200 samples

d. Mobile phase B (90:10 IPA:ACN + 10 mM Ammonium Formate + 0.1% Formic Acid)
1. Measure exactly 100 mL of acetonitrile in a pre-rinsed graduated cylinder and add them to the 1 L glass bottle.
2. Add 1 mL formic acid
3. Weight 0.630 g of ammonium formate and add them to the glass bottle
4. Sonicate for 40 min at 70ºC until all the ammonium formate is dissolved.
5. 1 L will last for around 200 samples

3.4 Pre-run sequence
a. Before starting the run inject the following:
1. 1× “No sample injection”
2. 5× Blank sample injection (methanol)
3. 2× QC-mix injection

4. 2× Citrate plasma injection

b. For the QC-mix, monitor the retention time, intensity, *S*/*N*, mass accuracy, and peak width (FWHM) of particular analytes (**Table 1**). Use the MassHunter Qualitative Analysis software for data processing. The acceptable ranges of the parameters are stored at <D>:\\<MassHunter\Methods\TEDDY methods>\TEDDY_QC-mix_default.XLS.
c. If those criteria are not met, the following actions should be considered:
(*i*) Replace the VanGuard pre-column and/or the UPLC column (if retention time shift >±2.5% and/or peak width expressed as FWHM increased >20%);
(*ii*) Clean the ion source (if intensity of particular analytes <80%);
(*iii*) Re-tune the mass spectrometer (mass accuracy of particular analytes >10 ppm).

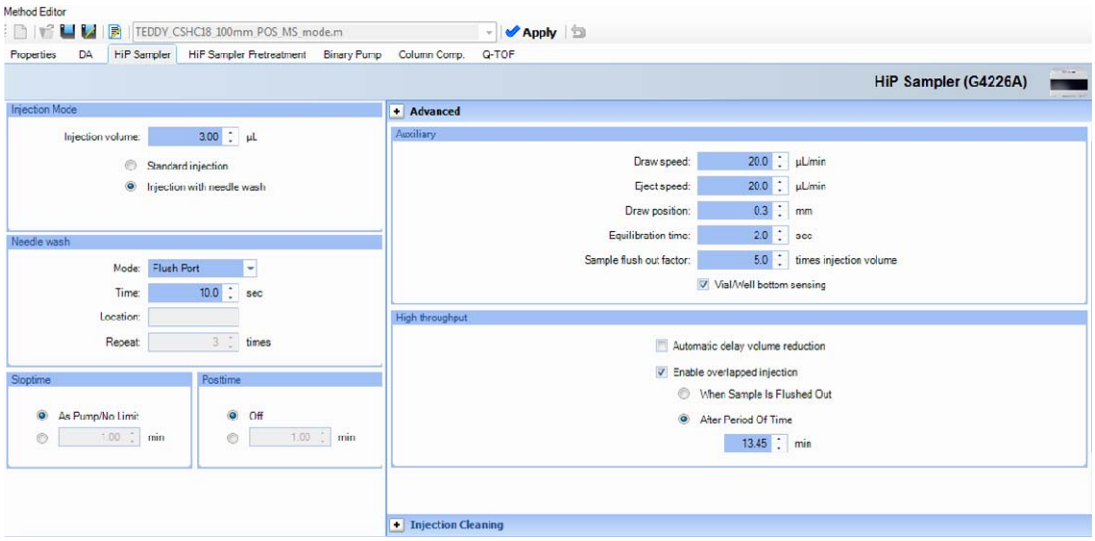
Table 1 Analytes of the QC-mix solution

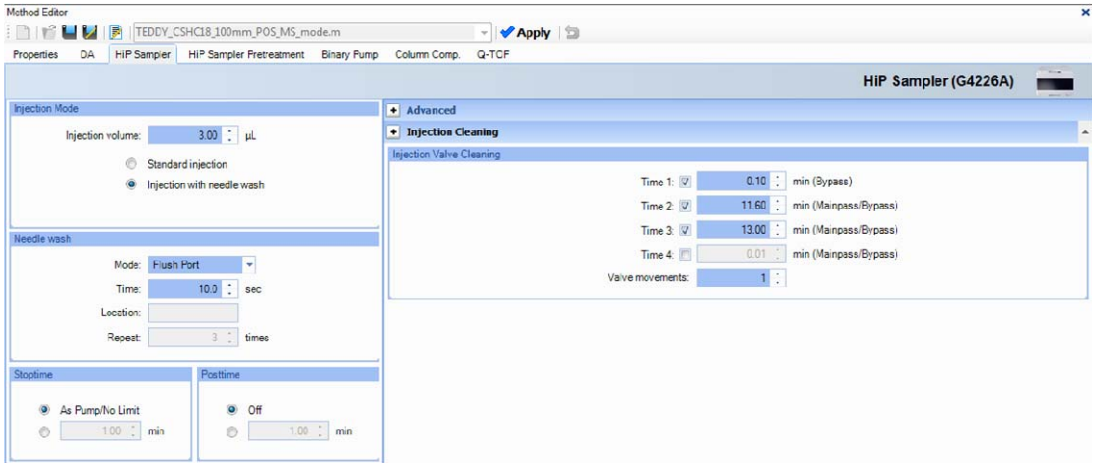
Common name	Formula	Exact mass	MS1 *m/z*	RT (min)
Cholesterol(d7)	C27H39D7O	393.3981	376.3994	4.734
PC(21:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	C51H90NO8P	875.6404	876.6507	6.321
19:0 cholesteryl ester	C46H82O2	666.6315	684.6693	11.556
TG(18:1(6Z)/18:1(9Z)/18:1(6Z))	C57H104O6	884.7833	902.8205	10.935
LPC(17:1(10Z)/0:0)	C25H50NO7P	507.3325	508.3416	1.311
LPC(13:0/0:0)	C21H44NO7P	453.2855	454.2948	0.818
PC(12:0/13:0)	C33H66NO8P	635.4526	636.4634	3.414
LPE(17:1(10Z)/0:0)	C22H44NO7P	465.2855	466.2935	1.390
PE(21:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	C48H84NO8P	833.5935	834.6031	6.496
PE(12:0/13:0)	C30H60NO8P	593.4057	594.4149	3.505
MG(18:1(9Z)/0:0/0:0)	C21H40O4	356.2927	374.3315	2.757

NOTE: Compare the profile of citrate plasma from a previously acquired sequence to that of a pre-run sequence. The variation within the TIC intensity must be <±15%.
NOTE: The backpressure should be within the range 500–580 bar at the beginning of each run [elution at 40% of the mobile phase (B)] and should not exceed the range 850–1000 bar [elution at 99% of the mobile phase (B)].
NOTE: If the initial backpressure is in the range of 580–725 bar replace the VanGuard pre-column. If the initial backpressure is still high even after the replacement of the VanGuard pre-column, use the new UPLC column.

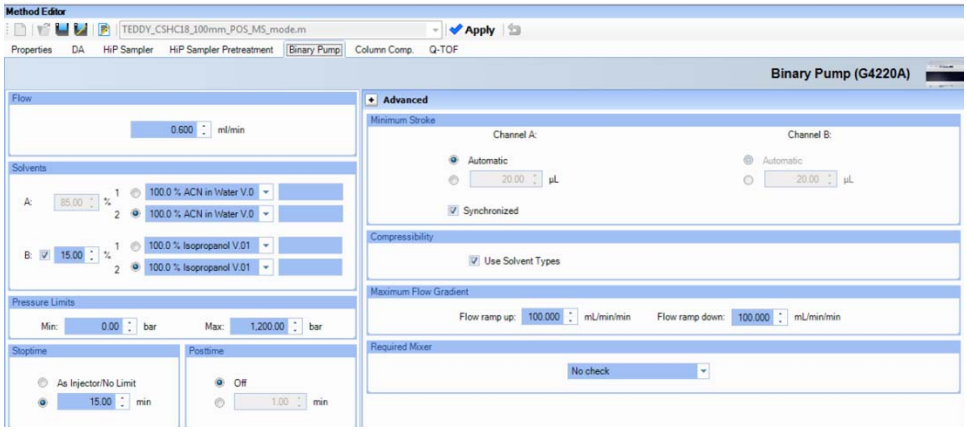
3.5 Lipid analysis method
a. There are four different methods for lipid analysis, under the folder <D>:\\<MassHunter\methods\TEDDY methods>\:
- Positive ion mode: TEDDY_CSHC18_100mm_POS_MS_mode
- Negative ion mode: TEDDY_CSHC18_100mm_NEG_MS_mode
- Positive ion MSMS mode: TEDDY_CSHC18_100mm_POS_MSMS _mode
- Negative ion MSMS mode: TEDDY_CSHC18_100mm_NEG_MSMS_mode

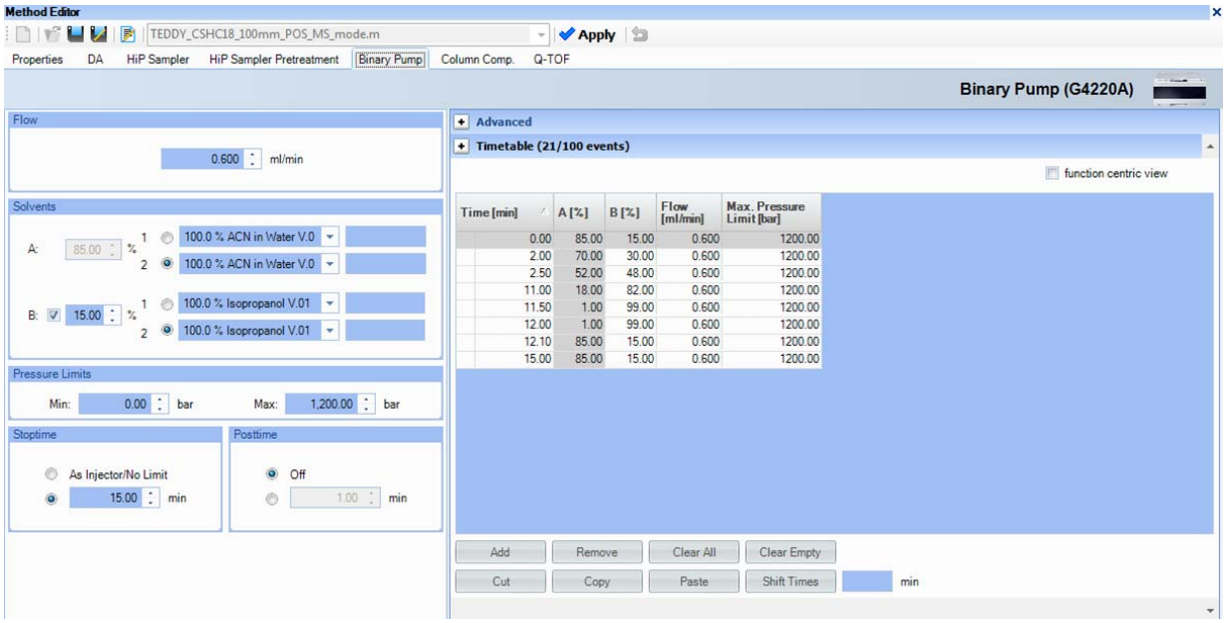
b. The autosampler, separation and column parameters for the lipid analysis method are as shown below:
- Autosampler


Method Editor
TEDDY_CSHC18_100mm_POS_MS_mode.m
Apply
Properties
DA
HiP Sampler
HiP Sampler Pretreatment
Binary Pump
Column Comp.
Q-TOF
HiP Sampler (G4226A)
Injection Mode
Injection volume: 3.00 µL
Standard injection
Injection with needle wash
Needle wash
Mode: Flush Port
Time: 10.0 sec
Location:
Repeat: 3 times
Stoptime
As Pump/No Limit
1.00 min
Posttime
Off
1.00 min
Advanced
Auxiliary
Draw speed: 20.0 µL/min
Eject speed: 20.0 µL/min
Draw position: 0.3 mm
Equilibration time: 2.0 sec
Sample flush out factor: 5.0 times injection volume
Vial/Well bottom sensing
High throughput
Automatic delay volume reduction
Enable overlapped injection
When Sample Is Flushed Out
After Period Of Time
13.45 min
Injection Cleaning

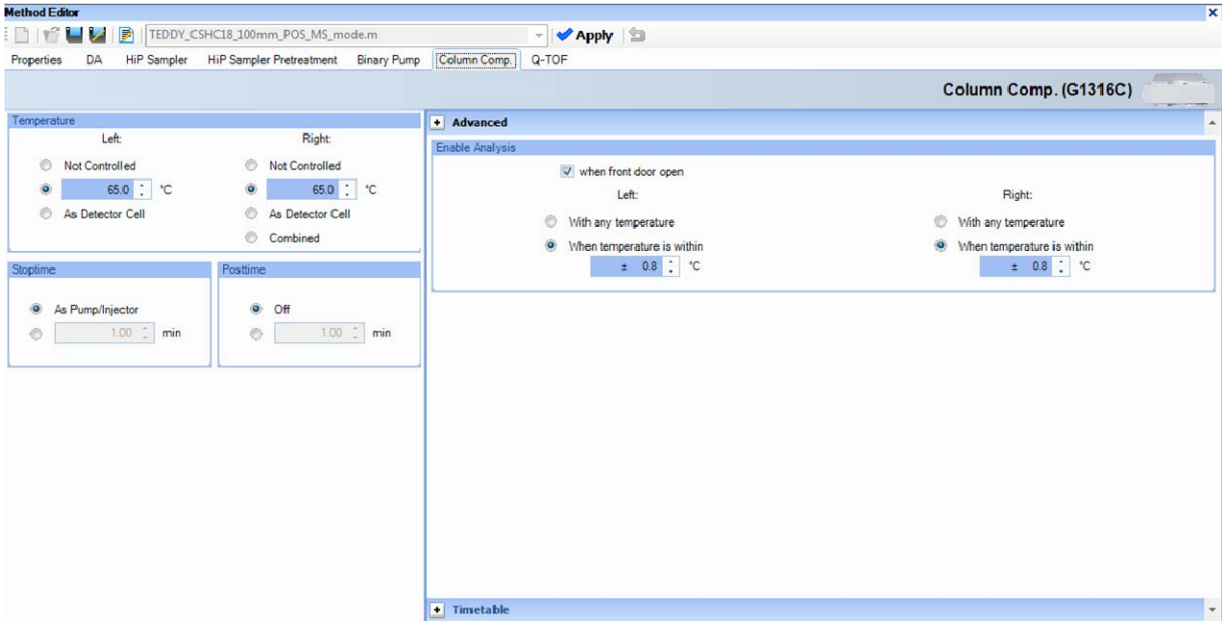

Method Editor
TEDDY_CSHC18_100mm_POS_MS_mode.m
Apply
Properties
DA
HiP Sampler
HiP Sampler Pretreatment
Binary Pump
Column Comp.
Q-TCF
HiP Sampler (G4226A)
Injection Mode
Injection volume: 3.00 µL
Standard injection
Injection with needle wash
Needle wash
Mode: Flush Port
Time: 10.0 sec
Location:
Repeat: 3 times
Stoptime
As Pump/No Limit
1.00 min
Posttime
Off
1.00 min
Advanced
Injection Cleaning
Injection Valve Cleaning
Time 1: 0.10 min (Bypass)
Time 2: 11.60 min (Mainpass/Bypass)
Time 3: 13.00 min (Mainpass/Bypass)
Time 4: 0.01 min (Mainpass/Bypass)
Valve movements: 1

- Binary pump

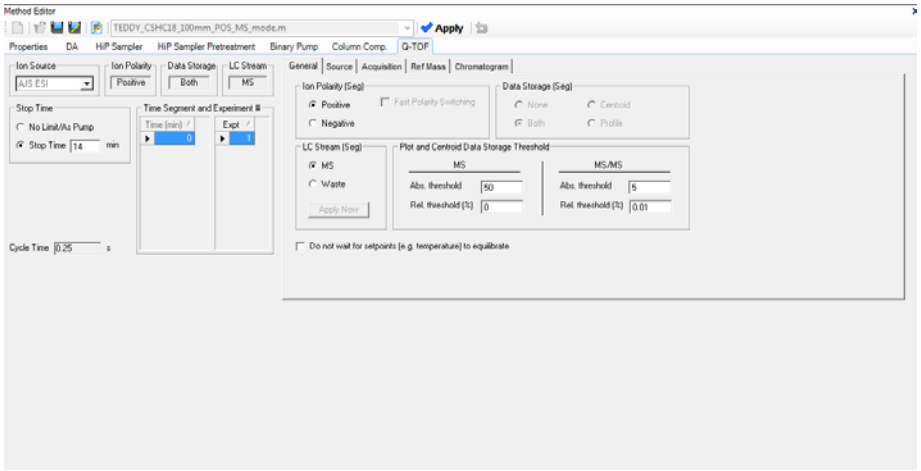

Method Editor
TEDDY_CSHC18_100mm_POS_MS_mode.m
Apply
Properties
DA
HiP Sampler
HiP Sampler Pretreatment
Binary Pump
Column Comp.
Q-TOF
Binary Pump (G4220A)
Flow
0.600 ml/min
Solvents
A: 85.00 %
1 100.0 % ACN in Water V.0
2 100.0 % ACN in Water V.0
B: 15.00 %
1 100.0 % Isopropanol V.01
2 100.0 % Isopropanol V.01
Pressure Limits
Min: 0.00 bar
Max: 1,200.00 bar
Stoptime
As Injector/No Limit
15.00 min
Posttime
Off
1.00 min
Advanced
Minimum Stroke
Channel A:
Channel B:
Automatic
20.00 µL
Synchronized
Compressibility
Use Solvent Types
Maximum Flow Gradient
Flow ramp up: 100.000 mL/min/min
Flow ramp down: 100.000 mL/min/min
Required Mixer
No check



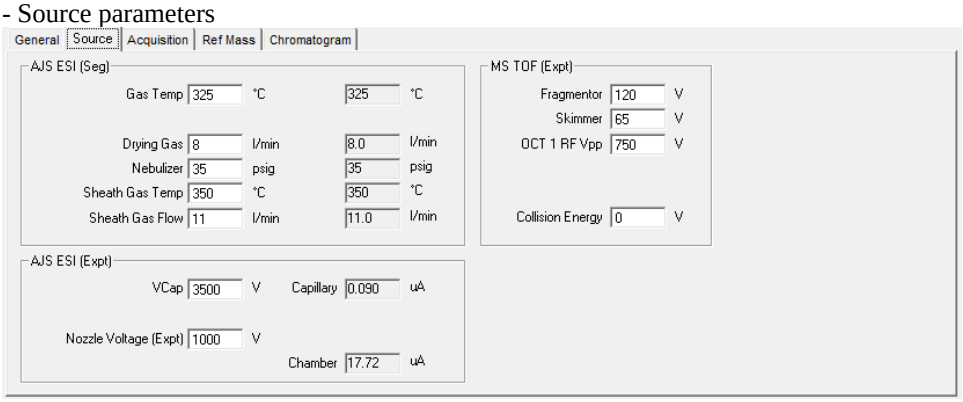
- Column manager
No timetable gradient



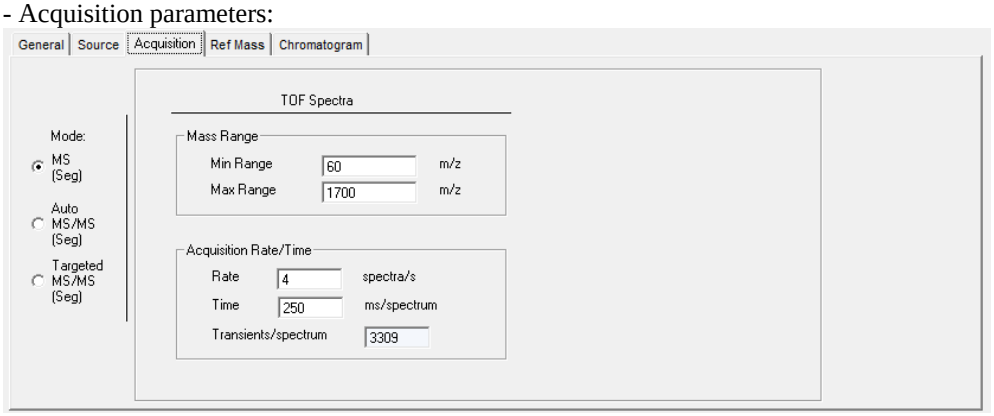
The MS conditions are the following:
3.5.1 Positive ion mode
- General parameters



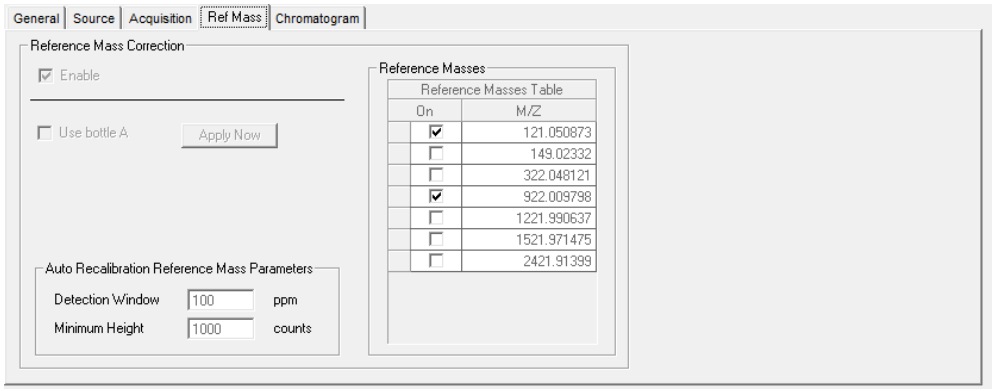
- Source parameters



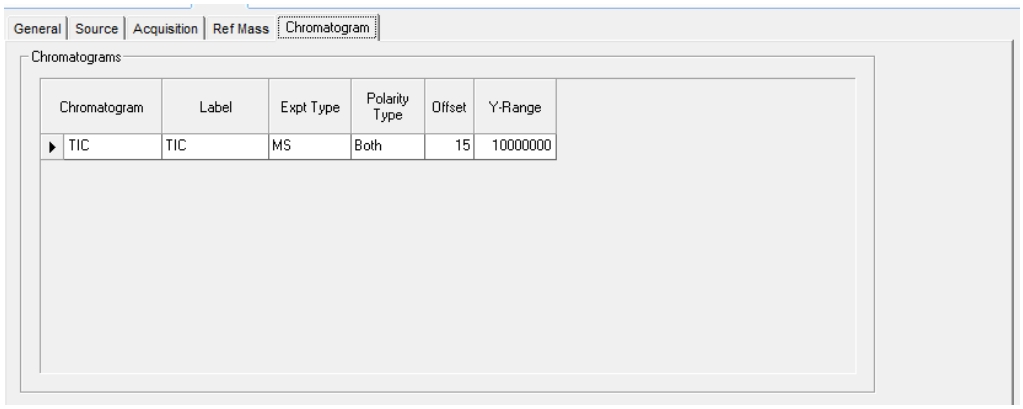
- Acquisition parameters:



- Ref Mass parameters



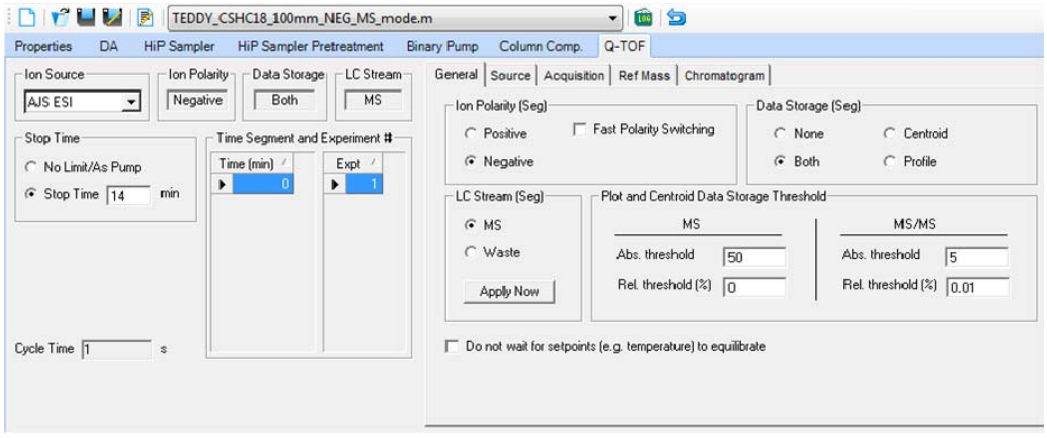
- Chromatogram parameters:



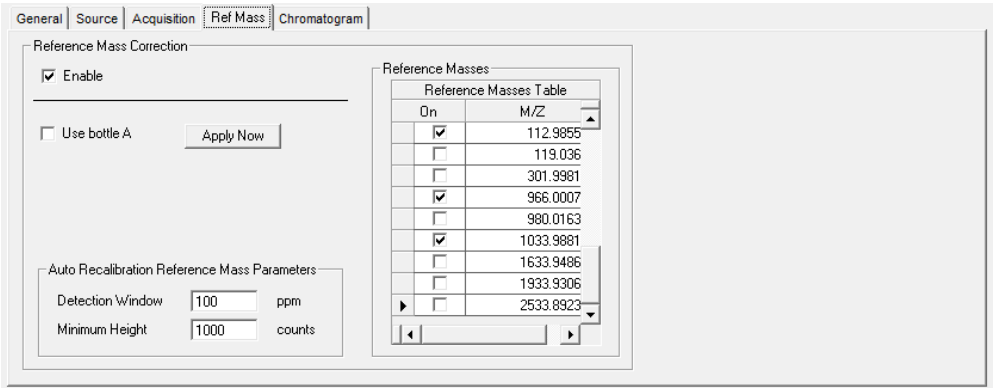
3.5.2 Negative ion mode

The parameters that vary from the positive mode are the following:

- General parameters



- Reference Mass parameters



3.6 Column storage

Use this procedure to avoid precipitation mobile-phase buffers on the column and in the system.

a. Flush column with 50% acetonitrile by setting the pump flow rate to 0.1 mL/min and increase the flow rate to 0.6 mL/min over 5 min; keep the column at this flow rate for 10 min.

b. Flush column with 100% acetonitrile by setting the pump flow rate to 0.1 mL/min and increase the flow rate to 0.6 mL/min over 5 min; keep he column at this flow rate for 10 min.

c. Remove the column from the system.

d. Store the column in the box until the next batch analysis. Add the story usage of the column.

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 lab 2.157 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 2.157 before monthly disposal. Other GC-TOF waste (rubber seals, O-rings etc.) can be disposed into regular waste.