Quality Control by HPLC-MS

1. Rationale/Aim

HPLC-MS is used as the main method for chemistry quality control (QC) to warrant that all chemogenomic compounds included in EUbOPEN chemogenomics library (CGL) meet the requirements of high quality set out in the criteria. Exact mass and purity of compounds will be assessed using an Agilent 1260 Infinity II LC System with a high sensitive Diode Array Detector, connected to a single Quadrupole LC/MS Mass Spectrometer. The system is equipped with an autosampler, which allows a medium-throughput pipeline consisting of sampling, measuring as well as documentation. The compounds pass the QC when having at least 95% purity and their identities are confirmed by an exact match between the observed and expected masses.

2. Experimental conditions

2.1 Key Requirement:

Compounds:

Compounds subjected to the measurement are prepared as stock solutions at 10 mM concentration in DMSO.

Instrument:

Agilent 1260 Infinity II LC System with Flexible Pump, Multisampler, Multicolumn Thermostat equipped with 4-column selection valve, a Diode Array Detector equipped with 60 mm MAX Light cell and a single Quadrupole LC/MS Mass Spectrometer.

Software:

OpenLAB CDS Chemstation Rev. C.01.10[201]

Method LC parameters:

Quat. Pump (G7104C)	
Mobile phase A	100.0% Water V.03 0.1% Formic acid (FA)
Mobile phase B	100.0% Acetonitrile (ACN) + 0.1% Formic acid (FA)
Flow rate	0.5 mL/min
Stop-/Posttime	7.00/ 2.00 min
Gradient timetable	0.00 min, 100% A 0.50 min, 100% A 6.00 min, 5% A
Multisampler (G7167A)	
Injection volume	0.5 µL
Needle wash	3 s in ACN + 0.1% FA
Draw/Eject speed	100.0/400.0 µL per min
Stop-/Posttime	As pump/ off
Multisampler Injector Program (G7167A)	
Draw	Draw 15 µL from location "3" with maximum speed using default offset
Eject	Eject 15 µL to sample with default speed using default offset
Draw	Draw 15 µL from sample with default speed using default offset
Eject	Eject 15 µL to sample with default speed using default offset
Inject	Inject
Column (G7116A)	
Agilent Poroshell 120 Bonus-RP, 2.1x100 mm, 2.7 µm, Serial# USFBM01961, Product# 695768-901T	
Temperature (left/right)	Not controlled
Diode Array Detector (DAD, G7117C)	
Wavelength/ Bandwidth	Signal A: 254/ 4 nm, Ref. 360/ 100 nm Signal B: 310/ 4 nm, Ref. 360/ 100 nm Signal C: 460/ 4 nM, no Ref. Signal D: 618/ 4 nM, no Ref.
Data rate	10 Hz
Store spectra	All
Range	190-640 nm
Mass Spectrometer Detector (MSD, G6125B)	
Tune File	Atunes.tun
Ionization Mode	API-ES, positive polarity
Nebulizer pressure	35 ps/g
Drying gas flow	12.0 L/min
Drying gas temperature	350 °C
Capillary voltage (positive+negative)	3000 V

Maintenance and Tuning:

1.) The mass spectrometry (MS) detector needs to be cleaned externally and tuned every week to ensure accurate performance.
   - For tuning: manually connect the tuning-mix solution bottle capillary with the MSD. Use the software OpenLAB CDS Chemstation (online) and go to the "MSD-Tune" window. Select tune file: "ATUNES.TUN" and tune each, positive and negative API-ES mode via clicking on "Tune" and "Check tune" afterwards. A report for each polarity will be created, which must be saved in the method by clicking "yes" twice.
   - For cleaning: open the housing of the MSD chamber. Clean the outer parts of the flow cell (Caution! Metal parts might be hot) and the spray capillary, using 70% v/v isopropanol with a dust-free cloth.

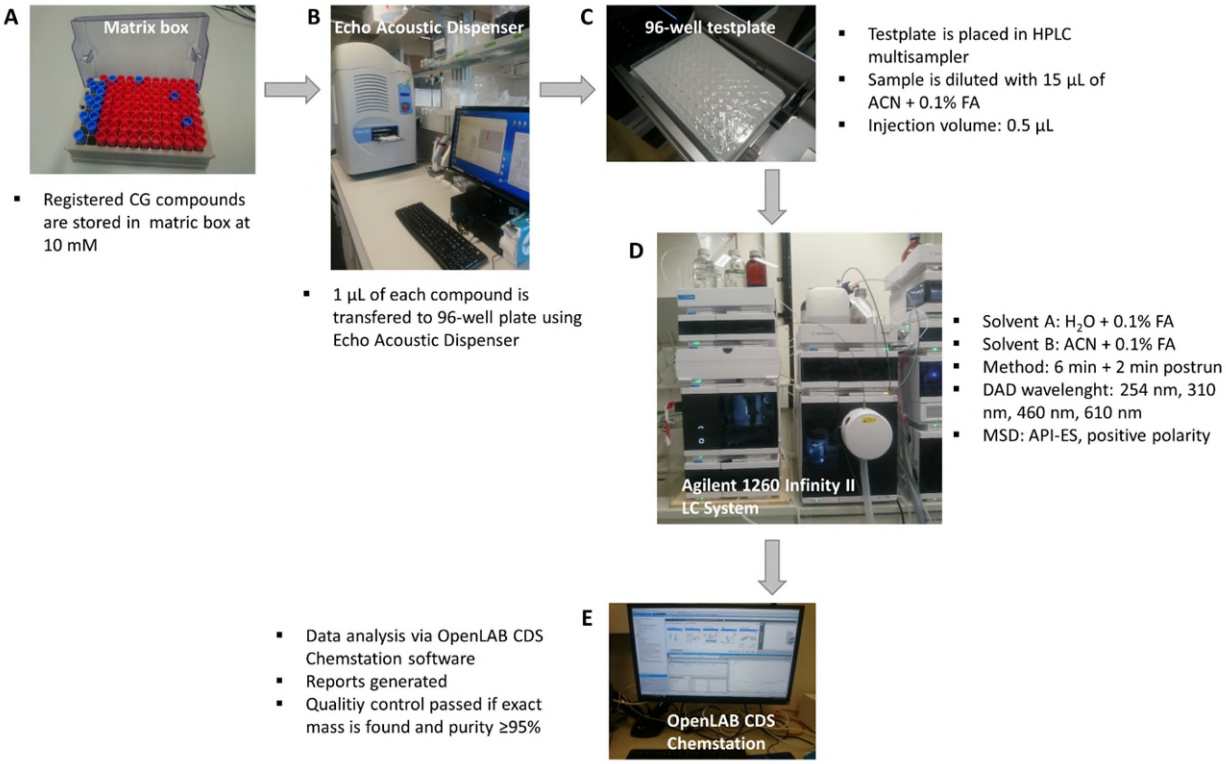
2.) Thorough cleaning of MSD-flow cell and adjustment of the spray needle needs to be done on a monthly basis.

2.2 Key resources table:

Reagent	Supplier	Code
Acetonitrile, anhydrous (max. 0.003% H_2O) ≥99.95%, HiPerSolv CHROMANORM®	VWR-Chemicals	83639.320
Formic acid, puriss. p.a., ACS reagent ≥98%	Merck	33015-1L-M
96 round well microplate (330 µL volume plate, V-bottom)	4titute®	4ti-0117
384-well armadillo plate	Thermo Fisher	AB3384

3. Protocol

3.1 Workflow



3.2 Protocol:

1) Chemogenomics compounds at 10 mM concentration in DMSO are transferred from the storage Matrix tubes into a 384-well Armadillo plate (source plate) compatible for Echo Acoustic dispenser.

2) Transfer 1 µL of each compound from the Armadillo source plate into each well of a 96-well, V-bottom microplate (assay plate) using Echo Acoustic Dispenser. Four assay plates are made per one 384-well source plate.

3) Seal the assay plates with thermal foil plate seal (do not use other types of plate seal to prevent sticking of the HPLC injector capillary).

4) Place the sealed assay plate in the HPLC multisampler container.

5) Refill the HPLC-vial at position 3 with acetonitrile + 0.1% FA solution. (This will be used to dilute compound samples in the assay plate according to our specific multisampler injection program – see method parameters).

6) Open software: "OpenLAB CDS Chemstation" (online mode).

7) Define sample location: go to "Method and Run Control" then "Sample Entry" and then choose the plate type "96 Agilent". Then, for each sample use the right mouse click to append the sample into the sample list.

8) In the sample list, fill in necessary information: 1) "sample name" - type in the compound name, 2) specify the method - choose "IMI_meth\SR_POS1-RP-0.5mL-min-MS POS SCAN-GRAD-7min_INJPROG.M" and 3) specify the injection volume - choose "default" for 0.5-µL injection, otherwise type in a desired injection volume.

9) Once the preparation of the sample list is done, click the option window (asterisk symbol on top right of the sample table), then go to "sequence parameters" and choose the shutdown options: "post-sequence comment" as "turn instrument standby".

10) Next, go to "Data file" and choose subdirectory EUbOPEN-DATA.

11) Then, 1) go to "Sequence summary" and select "print sequence summary report" and intelligent reporting, 2) define output file location (e.g. C:\SGC_FFM\) and specify output format (for our analysis procedure, the result files must be saved as PDF, CSV, TXT), and 3) specify names for the summary reports.

12) Once the preparation is complete, click "Add to queue" button. The instrument will start the measurement and the reports will be automatically created and can be retrieved from the data folder.

13) After the measurement, open "OpenLAB CDS Chemstation" (offline mode) and select "process data". Edit the integration events by selecting baseline as "classical" and adjusting the integration events such as slope sensitivity and reject area. Click "save" and confirm "copy change integration to method - yes".

14) To generate new reports, including all changes in integration, go to "sequence" (window on top of the desk) and choose "start reprocessing". All reports in the whole sequence will be updated.

15) The observed mass is calculated automatically from the TIC spectrum and included in the reports. For purity determination, the area of the peaks for each UV wavelength is calculated automatically. The maximum area of the peak (%) must be ≥95% for at least two wavelengths (254 nm and 310 nm) for the compound to pass the QC. The purity results are also included in the reports.