Agilent 1260 Infinity SFC/UHPLC System

User Manual 2020

Contents

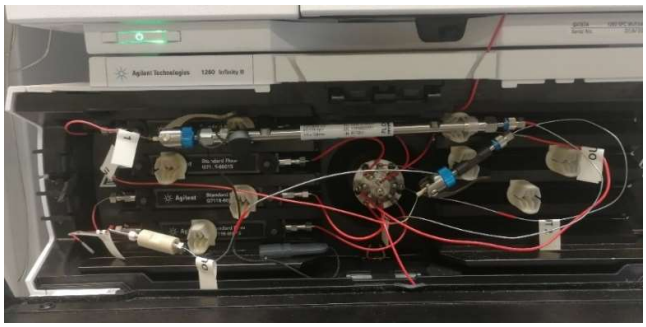
SAMPLE PREPARATION

Information on sample preparation equipment, consumables and solvents of MS-laboratory (O309) is presented in MS-laboratory general instructions (S:\chem-staff\INSTRUMENTS\MS\ MS_General_Instructions_2019_v1.pdf)

1. Weigh an exact amount of sample using semimicrobalance.
2. If possible, dissolve your compound into the same solvent you use as an eluent in your SFC/HPLC run.

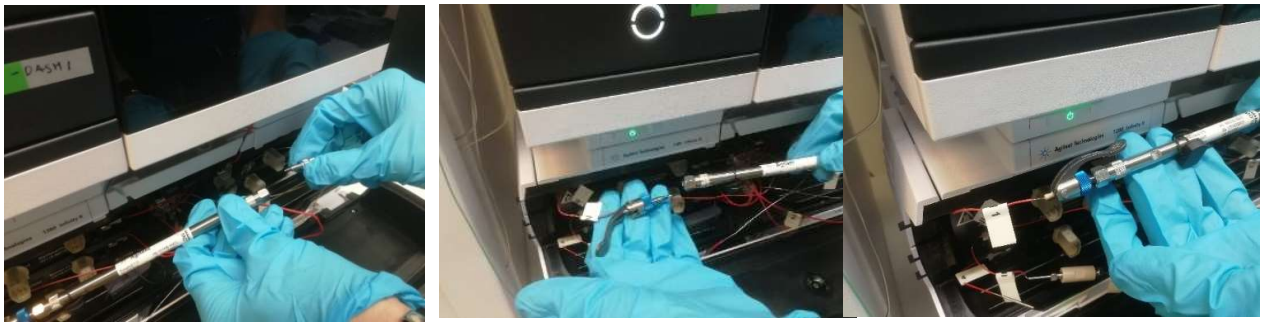
REMOVING/INSTALLING COLUMN

Column oven has four column positions, which are marked with numbers 1-4. Enter connections are marked by IN xx stickers and outputs by OUT xx stickers.



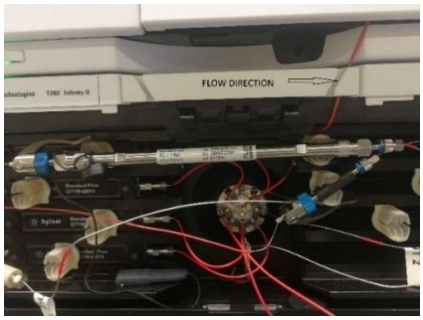
1. Before removing the column you have to run storage solvent in it (check instructions of the column).
2. Open connectors (traditional or quick connectors). When you have removed the column, install PTFE stoppers (in column´s storage box) to both ends of the column and put the column back to its own storage box.
3. Mark in column box which solvent is used for storage.
4. Choose the column you want to use and read the column instructions carefully. Pay attention to which solvents (and additives) are allowed to be used with the chosen column.
5. If you use pre-column, install it in front of analytical column using adapters.

NOTE: With Agilent 1260 Infinity SFC/UHPLC system you are not allowed to use hexane as eluent! If your column has hexane inside as storage solvent, you must run to the column another organic solvent first (example 2-propanol) using another LC instrument.



Opening the connector, quick connector (left), connector with handle open (middle), handle closed (right)

6. Take the column you want to use and check flow direction from the column
7. Install contactors carefully so that contactor is straight and goes the right threads.



SFC/UHPLC SOLVENTS

Check the solvents for HPLC and SFC *modifier*. Use only LC-MS grade solvents found in MS laboratory O309. Solvent lines are marked for SFC as B1 and B2 and for HPLC as A1, A2, B1 and B2. Make sure that solvent lines are not mixed. Make sure that there is no air bubbles in filter. If needed sonicate solvent prior to use.

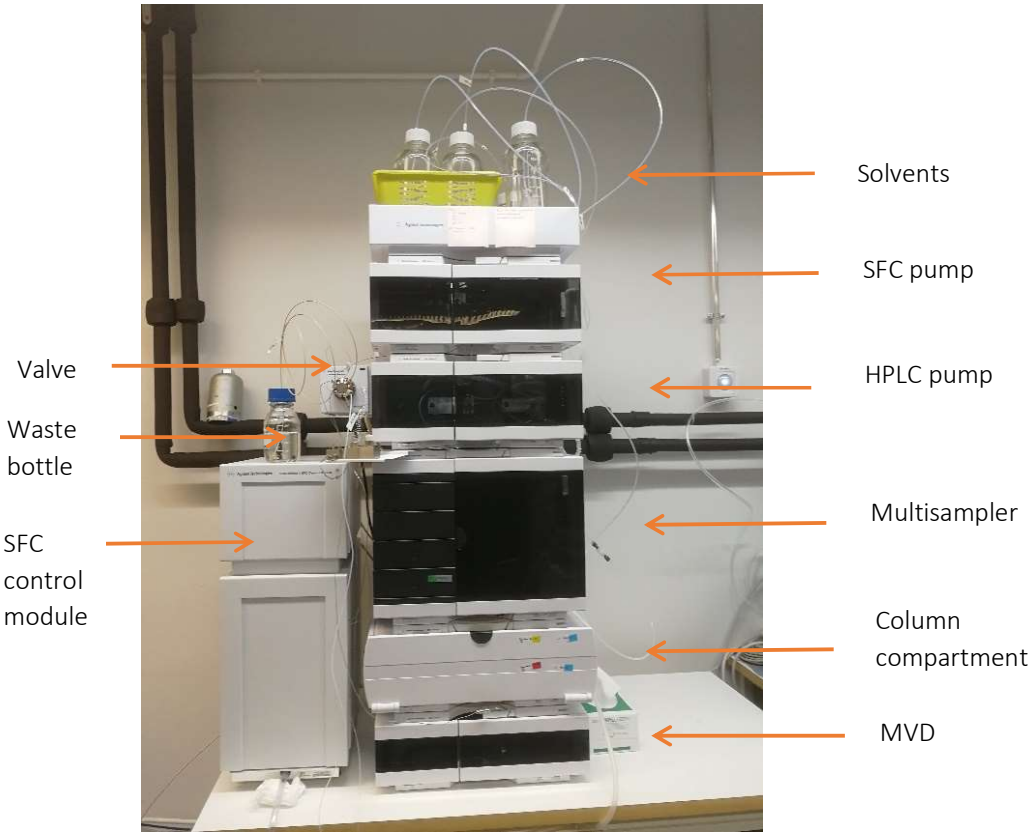
Solvents should be changed if instrument has been long time unused or solvents are old (check the date in bottles).

1. Open solvent bottle and place sinter (filter) into decanter glass for bottle cleaning and protect it from dust.
2. Discard old solvent in fume hood, wash bottle and let it dry.
3. Rinse bottle few times with small amount of solvent you wish to put in bottle.
4. Add solvent and mark date and solvent in bottle.
5. Shake filter in bottle to remove possible air bubbles.
6. When you have changed or added solvent in bottle, run clean solvent to solvent lines (purge)
7. Update *Bottle filling* in software by clicking status icons of HPLC and SFC with right mouse button.

STARTING Agilent 1260 Infinity SFC/UHPLC system

1. Open main valve for CO_2 cylinder (not necessary for UHPLC mode).
2. Switch on *SFC control module,* pumps, column oven, autosampler, and detector).

NOTE: Make sure that waste bottle has some solvent in it.

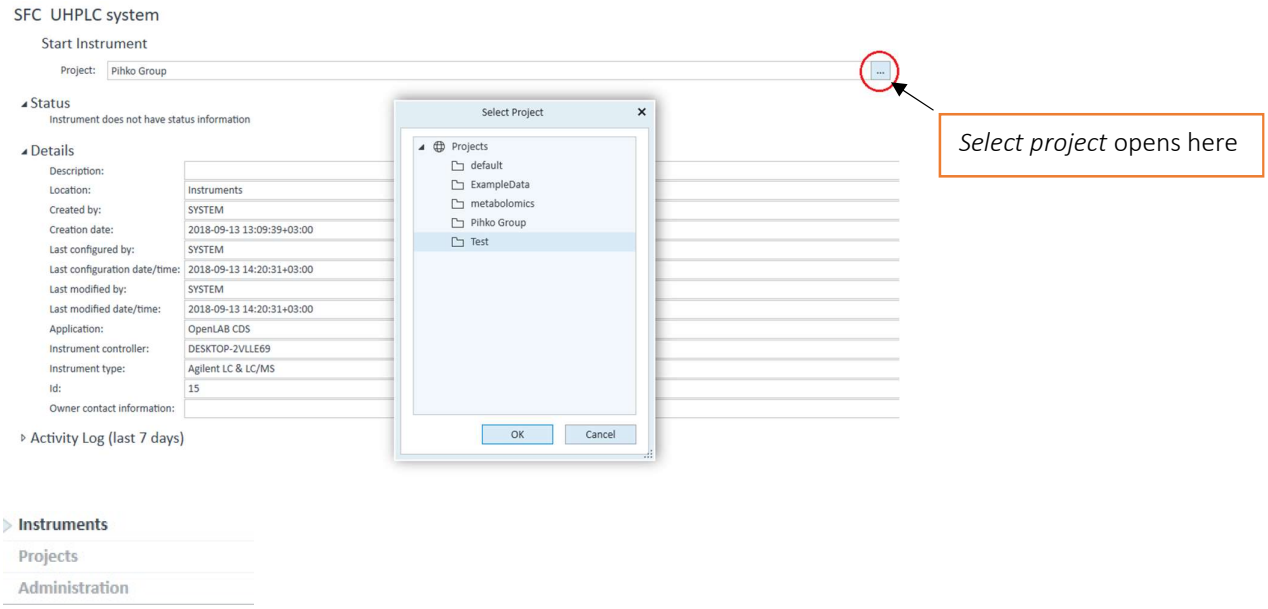


Agilent 1260 SFC/UHPLC system

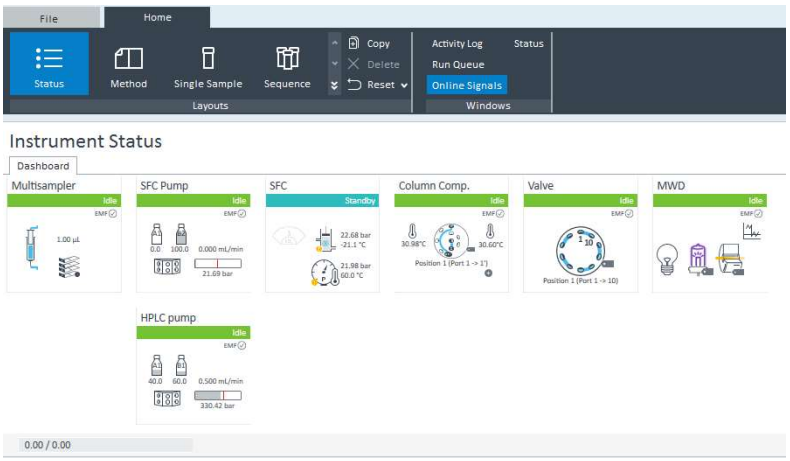
OPENING SOFTWARE

1. Open control panel by clicking CP icon 

2. Open *Instruments* -slide (in left corner) and choose the project you want to work with. Press ok and continue pressing *Launch* 



3. Open *Status* slide and *Instrument status* window, press *instrument on* and wait instrument components to get green status color.

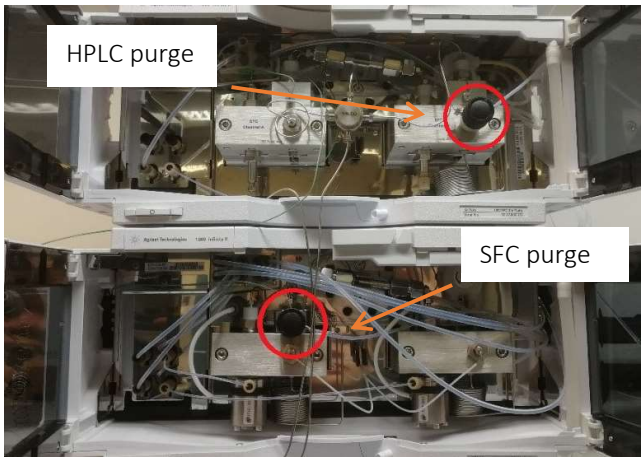


NOTE: When you do air purge and equilibrate the column the MWD should switch off (MWD icon`s off button). Finally, switch on detector in good time before the measurement is going to start, so it has time to heat up properly.

NOTE: Before measuring on HPLC mode, switch SFC module to Standby status. (right mouse click -> control -> standby).

CLEANING AND AIR PURGE FOR SOLVENT LINE

Before starting measurements you need to purge and clean solvent lines (remove previous solvent and air bubbles), which you are about to use.



1. Open purge for SFC or HPLC pump. (Figure 12).
2. Open *Method* menu and set for solvent 100% and flow rate 0.5 ml/min and *Send the current method to the instrument* pressing ⤓ If necessary flow rate can be increased until 2 ml/min.
3. Continue ~ 5min or until you don´t see any air bubbles in solvent line.
4. Repeat to all solvents you are about to use.
5. Close purge when you are finished.

EQUILIBRATION OF THE COLUMN

HPLC

1. Run first organic solvent (for example MeOH), by setting for HPLC MeOH 100% and flow rate to 0.2 ml/min → *Send the current method to the instrument* ⤓
2. Start to gradually add second eluent (max 5 % at time), until you reach target eluent ratio.
3. Increase flow rate gradually until you reach target flow rate.
4. Follow HPLC pump pressure and let it stabilize (10-20 min).

SFC

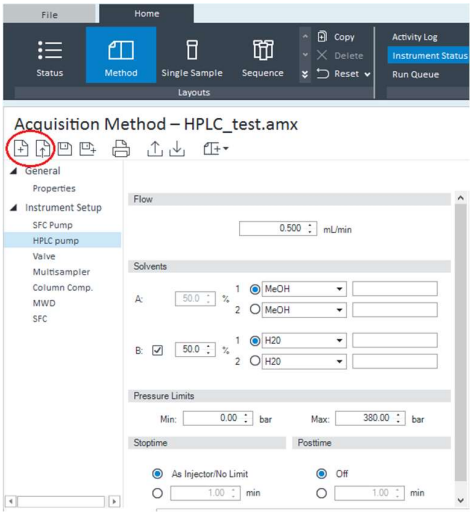
1. Run organic solvent first to column (for example MeOH), by setting for SFC MeOH 100% ja flow rate 0.2 ml/min → *Send the current method to the* ⤓ *instrument*
2. Set eluent ratio CO_2 90 % and modifier 10 %. Increase gradually modifier (max 5 % at a time), until you have reached target ratio.
3. Increase flow rate gradually (max 0.5 ml/min at a time) until you reach target flow rate.
4. Follow pressure at SFC pump and let it stabilize for 10-20 min.

OPENING/CREATING A METHOD

1. Open *Method slide* and open earlier created method or create a new one.
2. Set following parameters for your run. In *Properties* you can write general description of the method.

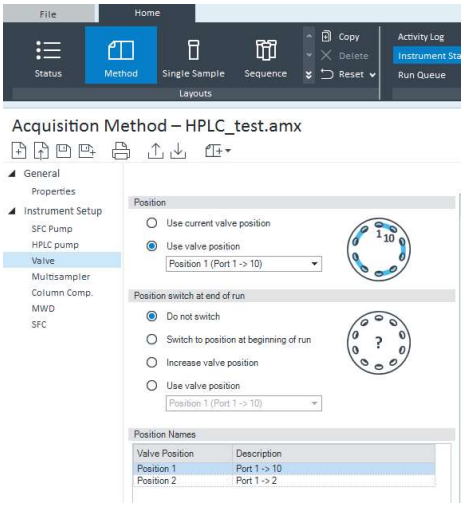
SFC pump

Flow rate	0.5 - 3 ml/min
Solvents	A1 = CO_2
	B1 = modifier X
	A2 = modifier X
	B2 = modifier X
Min and max pressure	0 bar and 300-500 bar (check from column)
Stoptime	As Injector / No Limit
Posttime	Off
Timetable	Create gradient here



HPLC pump

Flow rate	for example 0.2 – 0.5 ml/min
Solvents	A1= X, A2 = X, B1 =X, B2 = X
Min and max pressure	0 bar and 300-400 bar (check from column)
Stoptime	As Injector / No Limit
Posttime	Off



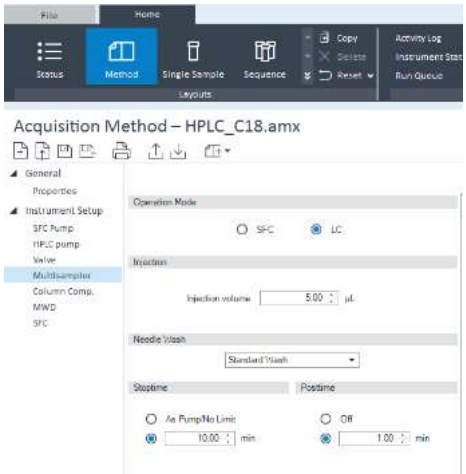
NOTE: If you create HPLC method, set SFC pump flow rate to 0 ml/min

Valve

HPLC	position 1
SFC	position 2

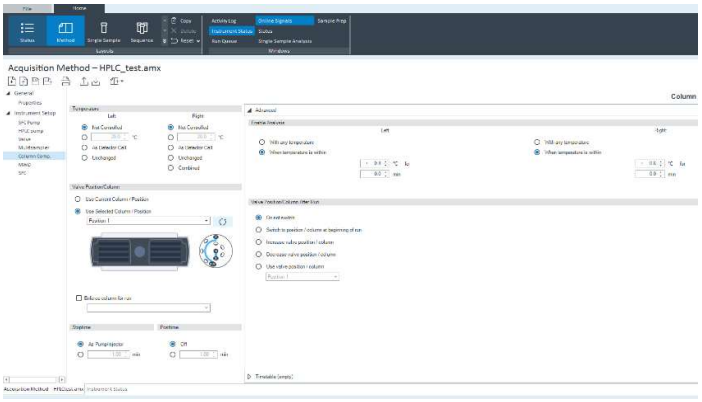
Multisampler

Operation mode	SFC or LC
Injection	for example 3 µl
Needle wash	Standard wash
Stoptime	for example 15 min
Posttime	for example 2 min



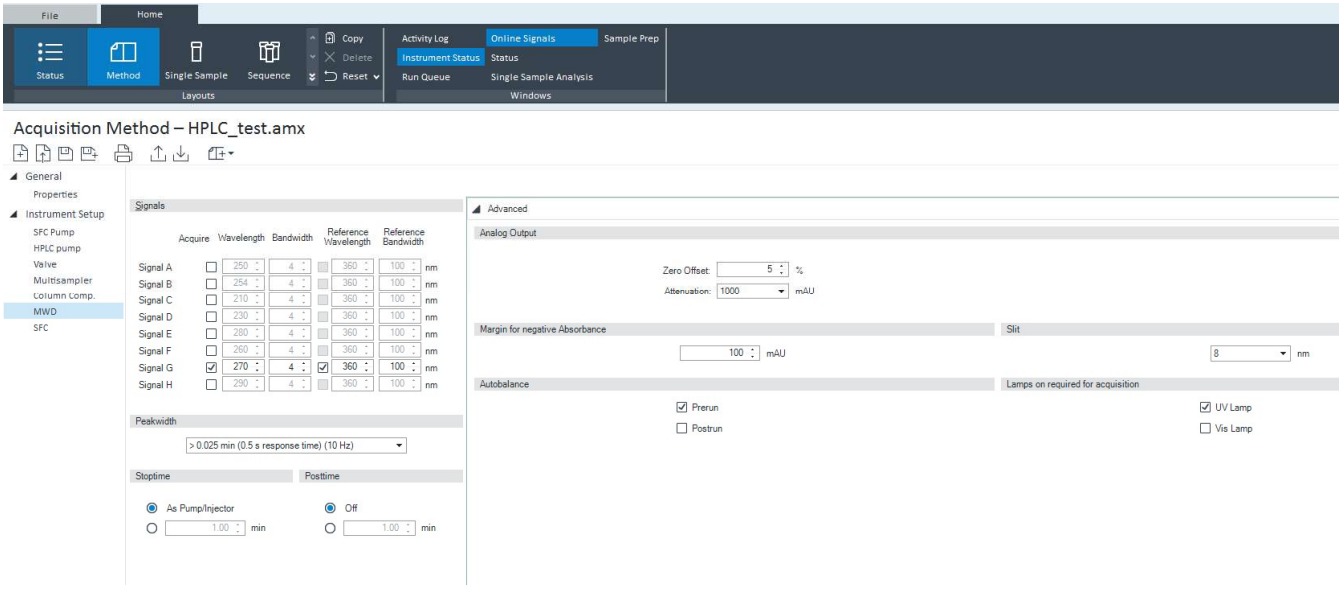
Column compartment

Temperature	Not controlled or 30 – 50 °C
Stoptime	As Pump/Injector
Posttime	Off
Valve Position/Column	Use selected



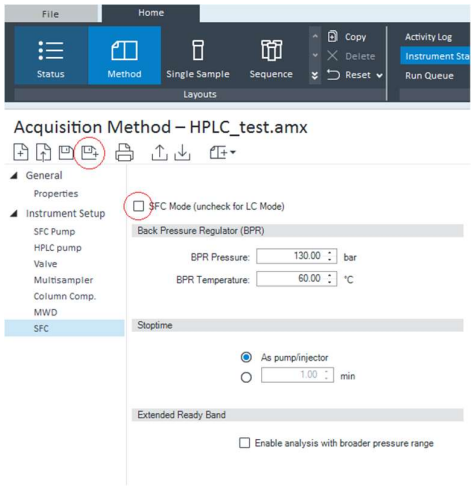
MVD –detektori

Signal	Choose correct wavelengths
Stoptime	As Pump/Injector
Posttime	Off



SFC

SFC mode	Choose when you measure on SFC mode
Back Pressure Regulator (BPR)	BPR Pressure for example 140 bar
BPR Temperature	for example 60 °C

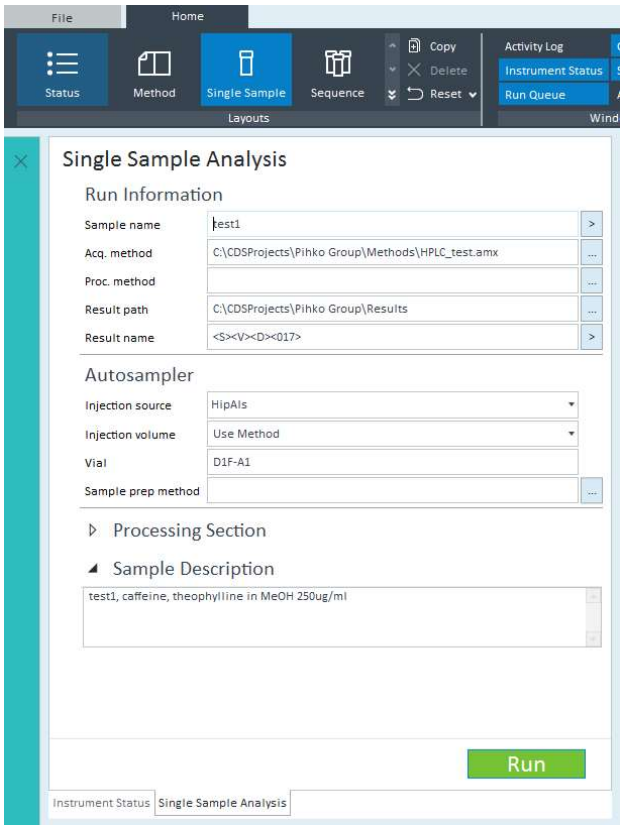


NOTE: When you measure on HPLC mode, please remember to check that *SFC Mode* is not active.

Finally, save your method clicking *Save the current Acquisition method as a new file.*

SAMPLE RUN

Single Sample



1. Open *Single sample* slide and fill following details:

   - Sample name (short)
   - Location in autosampler, for example D1F-A1, where:

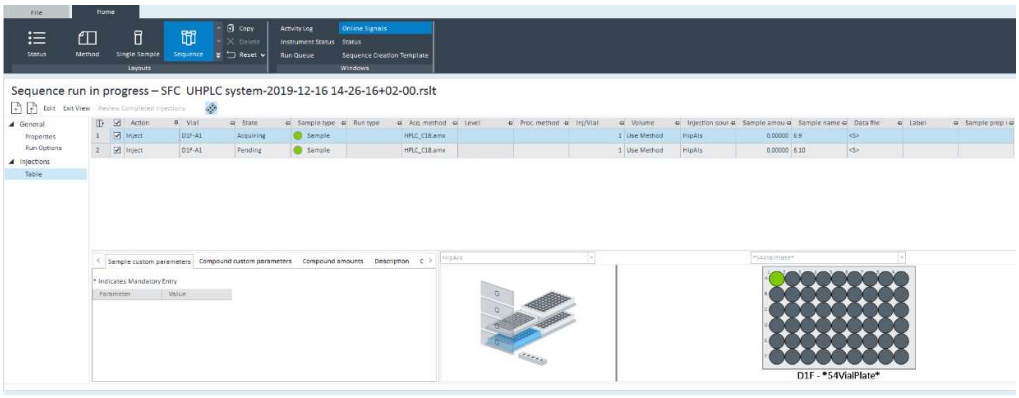
     D = lower sledge in autosampler
     F = front tray
     B = back tray B
     A1 = location of sample vial
   - Description of the sample
   - Run time (which is set in method)

Start acquisition by pressing Run

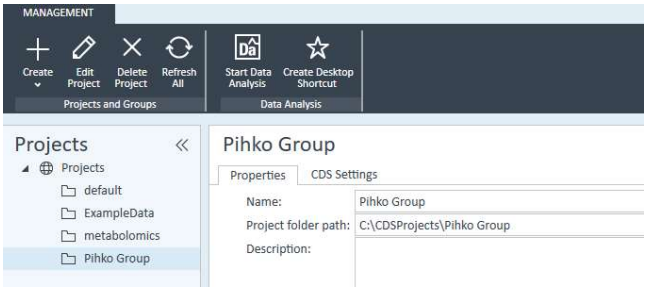
If necessary you can stop run by clicking ■ icon

Multiple samples

1. Choose *Sequence* slide and add more lines (by clicking with mouse right button and choose *Add an injection line to the end)*
2. Choose location of your sample, method, name and information you need to *Data file*. Choose samples you want to run and start run by clicking Run



DATA ANALYSIS



1. Open Control Panel → *Projects* → Choose project → *Start Data Analysis.*

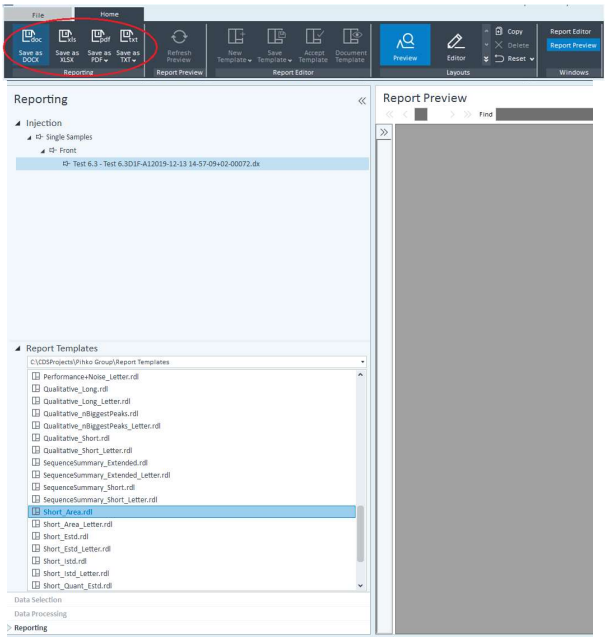
2. Data Analysis program opens. Open *Data selection* slide and click uppermost folder when injection list is updated.

3. Choose injections and click *Load Data*

4. Choose option you need (typically *GC/LC Quantitative*) click *Link Only/Link and process.*

5. *Link and process* integrates automatically, but *Link only* not. You can integrate manually clicking *Activate/Deactivate manual integration*.

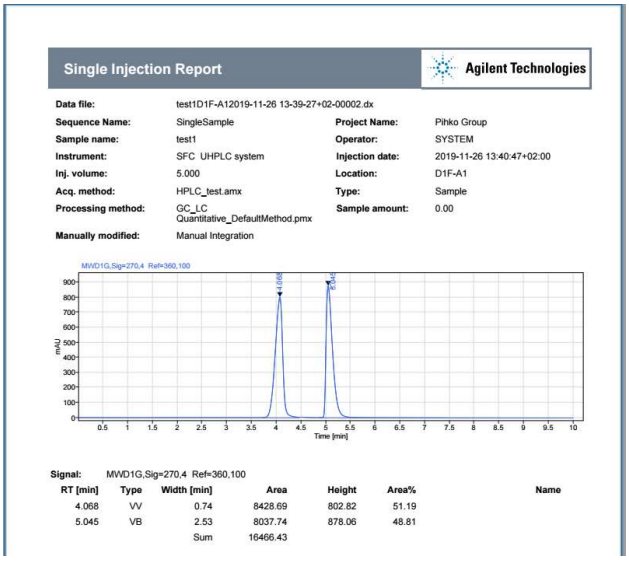
6. Finally, *Save all results.*



SAVING AND PRINTING THE RESULTS

1. Open *Reporting* → *Preview* → *Report templates* and choose report template.

2. Save report as .docx, .xlsx, .txt or .pdf



FINISHING MEASUREMENTS

SFC

1. Decrease SFC pump flow rate gradually (0.5 ml/min at a time), until flow rate is 0 ml/min.
2. Open HPLC method and continue according to HPLC instructions.

HPLC

1. Run storage solution to column (check column instructions) using flow rate of 0.5 ml/min.
2. Let column pressure to stabilize.
3. Decrease flow rate gradually.
4. Save method with flow rate of 0 ml/min.
5. Switch off instrument from *Status* view clicking Off.
6. Switch off software, PC and power of instruments.
7. Close main valve of CO_2 cylinder.

NOTE: If you don´t continue measurements on following day, disconnect column and put it in its box. Add in box a note with used strorage solvent and date.