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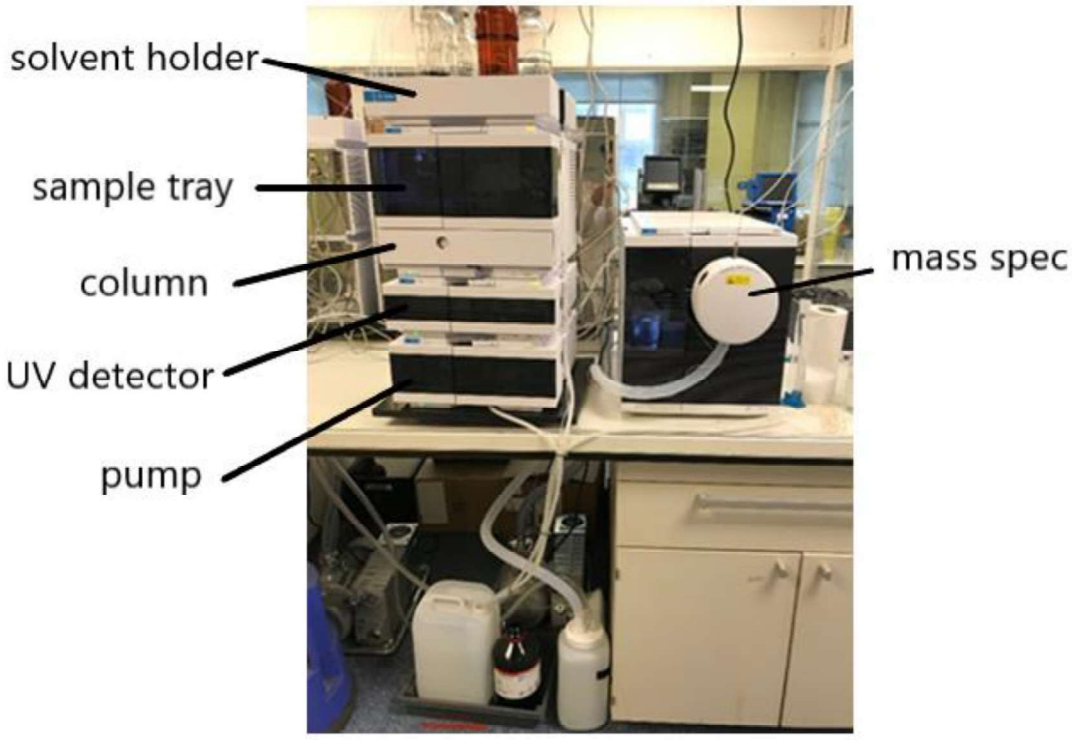
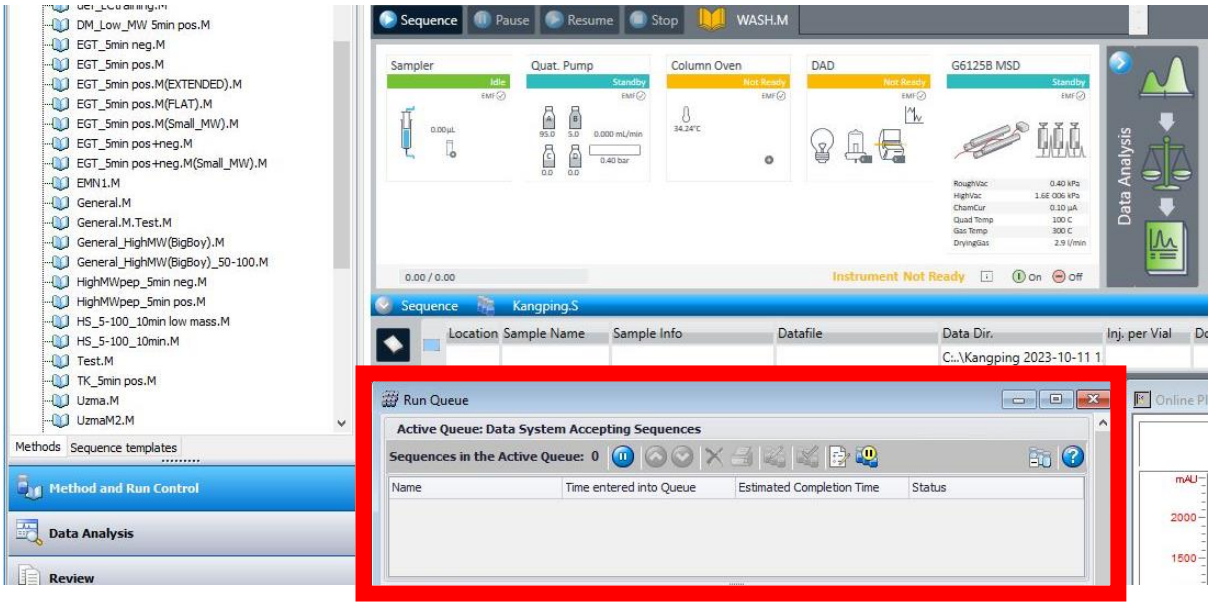
# G25 Analytical LCMS Operation Guide



# Running Sequence Injections

There are no sequences running or queued, as here, if the “Run Queue” box is empty:




If the LCMS is Free

1. Open your sequence template by double clicking on your sequence on the left, under method and run control, sequence template.
2. Enter sample details into the table: sample location (P1/P2 and vial location), sample name, method (if visible, click <browse> to select manually), injection volume (start with 0.5 µL).
3. Repeat for each sample.
    a. Right click and “append lines” to add lines, or copy previous lines.
    b. “Filldown” will automatically copy something into lines below.
4. Make sure that you have a wash at the end of your sequence table. There should be a vial containing methanol in P1-F11. Check it is full enough and make sure to run a 5-100% acetonitrile method, such as the EGT or WASH methods.
5. Click “Run”.

If the LCMS is busy:

1. Go to "Run Control > Queue Sequence..."
2. Open your sequence template and press OK.
3. Enter sample details as above.
4. Press “next”
    a. Ensure subdirectory is your initials.
    b. Shutdown procedure should have "Post-Sequence command/macro" ticked and set
    c. to "Turn Instrument Standby".
5. Click “Next” then “Add to back of queue”.

# Data Analysis

1. Open “ChemStation (offline)” from the desktop.
2. Find your subdirectory location.
3. Your sequence will be saved in its own entry.
4. Double-click to open an entry and load the data.
5. To view mass spectra from MSD TIC:
    a. Select “Spectrum Mode”
    b. Click peak apex (or any time) position button
    c. Click on the peak or time on the MSD TIC trace to view the mass spectrum at that time.
6. You can save a report as a PDF by right-clicking the entry and selecting “Preview Report”

# Common Problems

## Excessive Injection Amount

### Issue

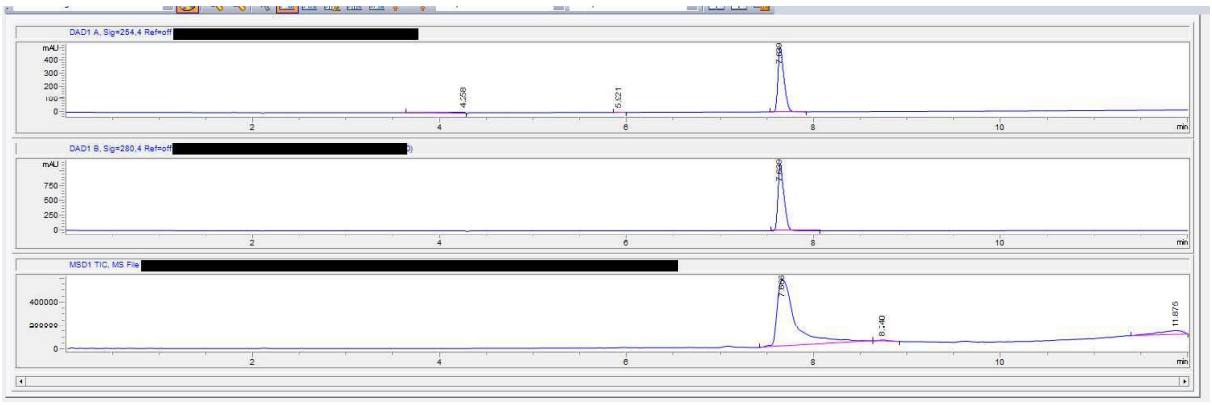
Too much sample was injected (i.e. over-injection), resulting in the analyte being visible on the MS trace throughout the chromatogram or carried over to subsequent runs.

### Cause

Either concentration of the sample was too high, or the injection volume was too large. Notice how the peak does not return to 0 in the MS TIC.

### Solution

Dilute the sample by taking one drop and dissolving in another 1-2 mL of LCMS solvent. Run the WASH.S sequence then a blank on your method to check if the peak has disappeared. If not, run the WASH_IPA.S sequence (ca. 1 h) and if that fails, try the WASH_MeOH+DCM.S sequence with HPLC dichloromethane connected to Line D. Some contaminants like trifluoroacetic acid are particularly sticky and will need to be leached out over time with several overnight runs at low flow rates.



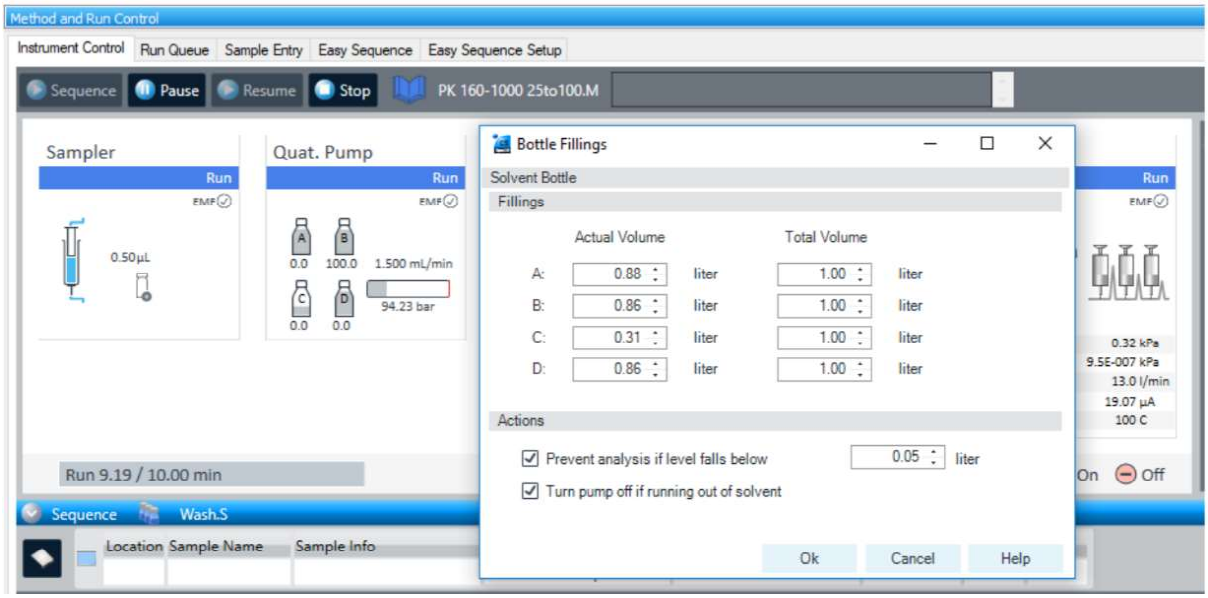
## Solvent is running low

Issue

Run has stopped as there is too little solvent.

Solution

Fill whichever/both bottles with LCMS grade solvent including 0.05% formic acid. Ask for help if you are unsure. Make sure to update the bottle fillings, as below, by left clicking the bottles under “Quat. Pump” and updating the relevant bottle.




## Error message

Issue

The pump has stopped, part of the screen has gone red and there is an error message.

Solution

Do not try to fix this! Get help from someone trained to fix the LCMS.

## Strange Peaks with 44 Da Difference

Issue

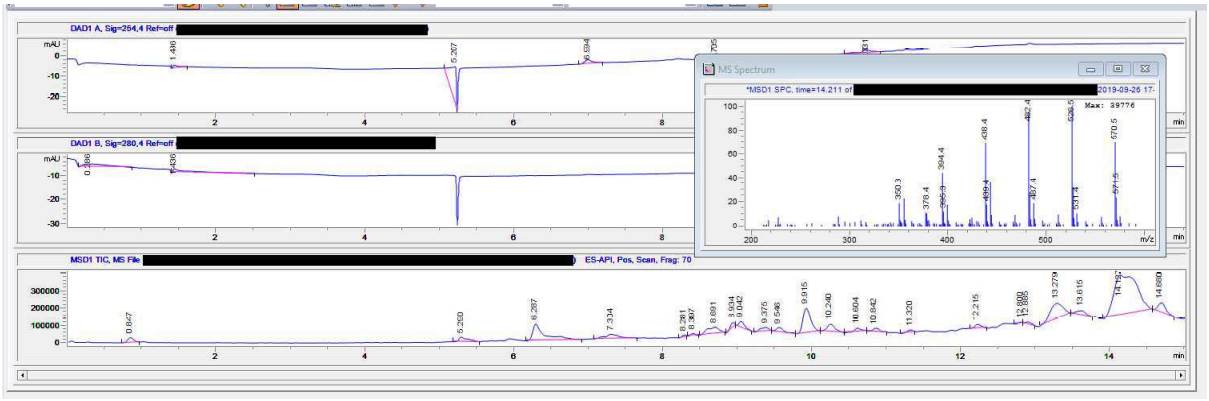
A large peak appears with many masses with a difference of 44 Da.

Cause

This is polyethylene glycol contamination (PEG). Sources include dirty solvent, detergent use, and plasticiser contamination from contact with plastics.

Solution

Do not wash bottles for LCMS solvent with detergent and avoid any plastic contact. Run a blank (no injection) then a solvent injection (i.e. only the sample solvent in a vial) to identify the source of contamination. If the blank is contaminated, then the system needs cleaning. If only the solvent injection is dirty, your injection solvent needs to be discarded.




## Peaks with 282 Da

Issue

A peak appears at high organic (i.e. >90% MeCN in $H_2O$), often broad, with mass of 282 Da.

Cause

Plasticiser contamination (probably oleamide) in your sample or a recent sample. This peak generally carries over several runs and is caused by improper solvent storage, typically after contact with plastic such as those in some vial caps or washing with acetone from a squirt bottle.

Solution

Wash the instrument by running the WASH_iPrOH.S sequence (this will take *ca*. 1 h) and test a blank. If this does not remove that contaminant, try the WASH_DCM/MeOH.S with Line D connected to dichloromethane.

Find the source of contamination based on previous experiments and inform the user. LCMS solvents should never be stored in vials with plastic film caps (glass vials with Teflon are generally compatible)

or typical lab glassware (e.g. round bottom flasks). Only store LCMS solvents in detergent-free glass bottles – a good choice is a 1 L bottle that came with LCMS grade solvent.