Waters UPLC-MS – Quick Start Guide

Polymer Facilities - MRL @ UCSB
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Reservations: Reserve with FBS, email Rachel to check about calibrations, etc.
Record number of samples on Log sheet and in FBS under consumables at end of reservation

Sample Preparation

1. Sample: ~200 pg/µL, soluble in H_2O, ACN, MeOH

Starting the MS:

1. Open MassLynx program, if not already open
    a. Check system status (Check for Green Lights!!!)
        i. Green light (ready)
        ii. Not scanning
    b. Open MS Tune Page and MS console ("A" page) from the MassLynx page, if not already open
    c. If the instrument says "Instrument in Source Standby", start up the instrument by pressing the startup button in on the MassLynx Shortcut page
        i. A pop up box will ask if you want to run Startup Sequence → "yes"
2. In MS console page, check that the appropriate calibration is loaded
    a. Go to Xevo G2-XS ToF heading on left side of page
    b. Click + button next to Xevo heading
    c. Click Intellistart
        i. Click Configure at top of page and make sure in Normal mode
        ii. Check box next to 'Use Calibration Profile'
            1. White Start button on right side of page
            2. Load appropriate calibration (Typically, NaI_2000)
    d. Do not change or check anything else
    e. Ask Rachel if you have questions about calibration/optimization
3. In Tune page, choose +ve or –ve mode, sensitivity for UPLC-MS
    a. ES tab and settings
        i. Voltages
            1. Capillary Voltage
                a. Enhances or suppresses ion density by supplying excess charge to droplets
                b. 0.5 – 3.0 kV (no higher than 2.0kV if in negative mode)
            2. Sample cone
                a. Helps draw ions into the first vacuum region
                b. 10 – 50 kV
            3. Source offset (80 kV – Do not change)
        ii. Temperature
            1. Source: 110-125C, change sparingly, as source temperature takes time to settle
            2. Desolvation: 200-400C for direct infusions
        iii. Gas Flow
            1. Cone (Helps reduce adduct ions and keep the sample cone clean)
                a. 20 – 50 L/hr
            2. Desolvation (Nitrogen)
                a. 1000 L/hr when running the UPLC
    b. Instrument tab
        i. Collision energy: used if you want to fragment sample more, can set for single energy, ramp, or multiple energies. Default (lowest energy) is 6
        ii. Target enhancement - off
    c. Fluidics tab

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i. Sample flow control- should be in LC position
ii. Lockspray flow control – no need to change
iii. Sprayer position – sample

Starting the UPLC:

1. Make sure all solvent bottles to be used are full
2. Open the "A" page to adjust UPLC flow
    a. Click on the Quaternary Solvent Manager on the left hand side
    b. Adjust the pump flow to 0.1 mL/min to begin flow, and set your A:B ratios (H_2O:ACN)
    c. For rinsing, B (ACN) typically is 50-80%
    d. For loop injections, flow is 0.3 mL/min at 70:30 H_2O:ACN
    e. For column injections, flow is 0.5-0.6 mL/min, gradient program
3. Let the system equilibrate for ~5 minutes, making sure delta PSI is low (20 PSI or less)

Creating and Running a Sequence:

1. Open the MassLynx page
2. Load your samples into the sampling tray
    a. Samples should be in a 2mL wide top screw-top vial with septa
3. Enter your samples into the spreadsheet starting on line 10
    a. File name: Date MMDDYY Initials Sample LC-run number
    b. Default file name, add additional information
    c. MS File: choose from loop inject (1.5 min) or column inject (15 min) and appropriate mass range
    d. Inlet File: choose loop inject + or – method (2.5 min) or column inject + or – (15 min)
    e. Bottle: Enter using the format Tray number:position (example 1:31 means tray 1 position 31)
    f. Injection Volume: 1-5 uL (max: 10 uL)
    g. Run a blank as the first run and in between samples to ensure the needle and lines are fully rinsed
4. For a long list of samples use the "Insert/Filldown wizard"
5. Save sequence
    a. Go to "File", then "Save as"
    b. Save as filedate: YYYYMMDD.SPL
6. Make sure the tray is replaced correctly or else you will not be able to run your sequence
7. Highlight the rows you wish to run
8. Click on the "Start" button (blue arrow/go button) to begin analyzing your samples
9. If you have modified the sequence, you will be asked whether to save changes, choose yes
10. Pop up box will appear
    a. Left side of pop up box: make sure acquire sample data is checked
    b. Right side of pop up box: make sure correct sample lines are entered
    c. Click ok to run
11. Additional samples can be entered into spreadsheet and added to queue while running, by pressing the start button (make sure autosampler is not injecting when adding to tray!)
12. Add "HPLC Shutdown" line to the queue after your sequence
13. **Fill out the log sheet!**

Processing Data from Sequence:

1. Click on the MassLynx application at the bottom of the screen
2. This brings up the MassLynx window
3. Click on the Chromatogram tab to open the acquired spectrum
4. Choose the correct file (.raw) for the data acquisition from the Chromatogram Data Browser
5. Select the region of interest for the acquisition by clicking with the Right mouse button and dragging.

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6. When you release the right button, the spectrum will appear
7. Use the left mouse button to select the region of interest for the spectrum.