Dartmouth College
Instrument core facility

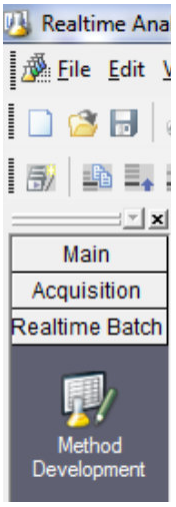
Overview

This set of instructions will guide you through the process of obtaining the nominal mass of your purified, isolated compound via the Shimadzu 8030 LCMSMS. Complete manuals from Shimadzu are available on the software LabSolutions and online.

Preparations

In your lab

- Prepare your sample at approximately 10uM in MS grade MeOH
- The 2ml autosampler vial must have >0.4ml of your solution
- Know your exact mass of your compound. Ie chlorobenzene's exact mass is 112.008 g/mol (whereas it's molecular weight is 112.556 g/mol). Here's a good calculator: sisweb.com/mstools/isotope.htm



At the instrument

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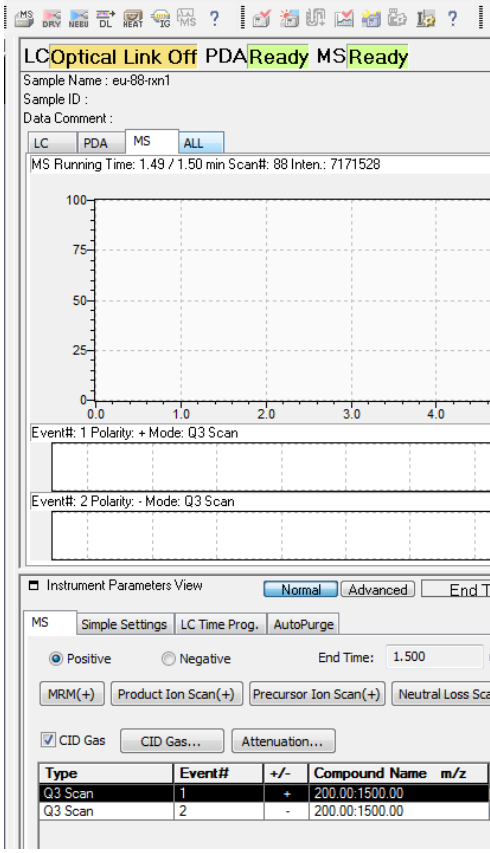
- Check eluents. Filtered tube ends must be submerged.
- Open/check gases. Both gas cylinders must have >100psi.
- Power on all components.
- If not already open, click LabSolutions, OK (no password) and from Instrument options, choose LCMS-PDA

To run MeOH blanks

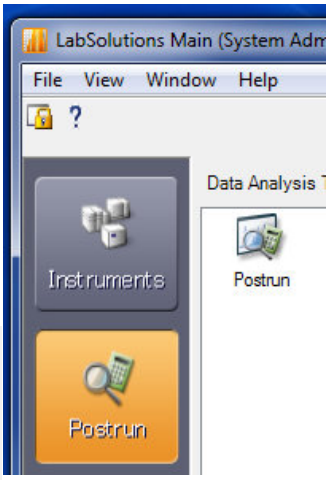
- Go to File/Open Batch File. File up until you find the BATCH folder and choose MeOH PreRun batch. (If you don't see Open Batch File then find the Assistant Bar (leftmost column of icons) then choose Realtime Batch to get to the File/Open Batch File option.)
- The batch should have 3 injections of methanol programmed.
- Open autosampler and confirm that the vial has sufficient MeOH.
- To set the method's m/z window so that your target will be encompassed
  - Click on Method Development icon in Assistant Bar, then Instrument Parameters (takes 5 seconds), then the MS tab to view the 3 Scans' Start and End m/z.
  - Edit both the and - Q3 Scan as necessary.
  - Go back to Realtime Batch by clicking on the

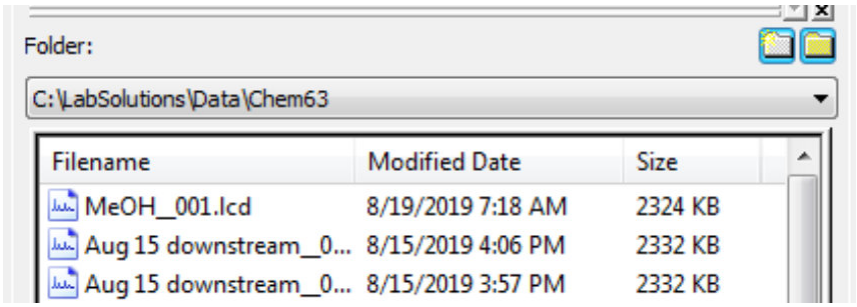


- Click the Batch Start to start the batch. Realize that pop-up boxes will warn if method or batch got changed. Click OK.
- It may take a few minutes for parameters to equilibrate before autosampler begins.

To view the blanks' data

- Open a Postrun from the main LabSolutions popup if one's not already open.
- Find Layout in the top file menu and choose Layout for Qualitative, Double click on a recent MeOH blank from browser to view TIC. The sample hits the mass spec detector around 0.2-0.4 minutes.
- Click the Average Spectrum icon then click on left part of peak, drag through peak and unclick on right side of peak.
- Click the Subtract Spectrum and similarly select some of the noise area.
- Review data. Intensity may be <50M for + and <IOM for - depending on many factors. Common contaminants from system, caps, etc are revealed. Note their m/z and intensity.





Flow Injection of your sannple (no column)

- Follow the steps below to inject the smallest amount of your sample and have your data saved in proper location.
  - Place your sample(s) securely in the Autosampler tray.
  - In Realtime Analysis, open batch called 'default.lcb' via File/Open Batch Folder.
  - Go to Batch/Settings/Folder tab to choose YOUR data folder, OK.
  - Edit the line(s) of the default batch: vial # and sample name. The method should be the generic FlowInjScanPosNeg.lcm. Start with 0.1ul sample size.
- Click the Start Batch green arrow.
- Flip over to Postrun to view your data just as the MeOH blanks were viewed. Consider what adducts may form in the positive and negative scans. Rerun with 1.0ul sample size if concentration is low

Data Report Generation

- In Postrun, have your data set open and the peak averaged and background subtracted.
- Change the threshold of m/z detection by right clicking on either Event scan and choose MS Data View Paramters/Spectrum Format, then check box for Relative Intensity Threshold and edit number to 10, 15 or 20. Apply, OK.
- Within the positive scan Event, right click and choose Register to Spectrum Process Table, OK. Do the same with the negative scan event.
- Find Data Report in the Assitant Bar and click on it. (takes a few seconds)
- Go to File/Open Report Format File and file up to find the folder called Sample (which resides in LabSolutions folder). Within Sample folder, choose MSMS/-dc_A_flowPosNeg.lsr.
- To change x-axis of MS Spectrum, right click, choose Properties/Setting Scale, edit Display Range x values, OK.
- Preview if desired. To save as pdf, click File/PDF Output.
- When finished, click the lower X in upper right corner to flip back to MS Data.

Return instrument to resting position

- Record your activity in the LCMS logbook, report any problems to ICF Manager.
- In Realtime Analysis, toggle components off, then put instrument 'to sleep'.
- Power off upper pump.
- Close Nitrogen gas.
- Remove your samples from the Autosampler.