

# **University of Waterloo Mass Spectrometry Facility (UWMSF)**

## **Waters Synapt G2Si HDMS Standard Operating Procedure (SOP)**

### **READ & UNDERSTAND THE ENTIRE SOP BEFORE BEGINNING**

Note: An SOP cannot cover all the needed information for proper instrument understanding and operation, or cover all scenarios; nor can an SOP eliminate the necessity of awareness, attention to details and most importantly using your training to allow successful completion of your project. Background information regarding ionization methods, ion separation, techniques and concepts as well as tips and tricks can be found on the UWMSF website:

<https://uwaterloo.ca/mass-spectrometry-facility/ms-tutorials>

Remember, the training you have received and the detailed protocol you have developed and documented will provide the guidance necessary to allow you to complete a successful sample analysis or project using this sophisticated instrument. Please refer to the instrument manuals for other background information, review literature, and always remember your training. When in doubt always ask Facility personnel before trying something outside of your training or prepared protocol.

This instrument can be used in high mass resolution MS and MS/MS modes with or without Travelling Wave Ion Mobility and because it has 2 collision cells, the range of experiments that can be performed are much more complex than with the Thermo Q-Exactive so this SOP is of necessity, very generic. The specifics necessary to run a successful project will be addressed during your training.

# University of Waterloo Mass Spectrometry Facility (UWMSF)

## Waters Synapt G2Si HDMS Standard Operating Procedure (SOP)

|                     |                                                                                                               |
|---------------------|---------------------------------------------------------------------------------------------------------------|
| Name                | Waters Synapt G2Si HDMS with I-class UPLC                                                                     |
| Description         | Hybrid Quadrupole-ToF LC/MS/MS system with ESI, APCI and Ion Mobility                                         |
| Location            | C2-268                                                                                                        |
| SOP created         | January 3rd, 2020                                                                                             |
| SOP author          | Richard W. Smith                                                                                              |
| SOP revised         | January 3rd, 2025                                                                                             |
| SOP revised by      | Dr. Yiming Xiao (YX) and Dr. Joshua Featherstone (JF)                                                         |
| SOP Location        | C2-268                                                                                                        |
| User Manual         | In LHS cabinet beside the instrument in C2-268                                                                |
| Equipment owner     | UWMSF                                                                                                         |
| Authorized Trainers | Dr. Yiming Xiao (YX) and Dr. Joshua Featherstone (JF)                                                         |
| Authorized Users    | Users who have completed the UWMSF Training Certification and all other relevant UW specified safety training |
|                     |                                                                                                               |



|                         |                                                                                                                                                                                                                                                                                  |
|-------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Significant Hazards     | High Voltage (up to 5kV AC/DC) and high temperatures (up to 350°C) are present in source and internal instrument components and power supplies.                                                                                                                                  |
| Administrative Controls | Can be used independently by users who have satisfied all training requirements described above. Other users must be accompanied by an authorized trainer. Instrument panels and interlocks should never be removed by users only by YX or JS when deemed necessary by YX or JS. |
| Engineering Controls    | Instrument is fitted with a protective housing and interlocks that prevent contact with high voltage/temperature components                                                                                                                                                      |
| PPE Required            | Gloves, eye protection, lab coats and closed toe shoes when appropriate                                                                                                                                                                                                          |

# University of Waterloo Mass Spectrometry Facility (UWMSF)

## Waters Synapt G2Si HDMS Standard Operating Procedure (SOP)

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                  |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Relevant MSDS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | See chemical inventory binder for relevant MSDS's                                                                                                                                                                                                                                                                                |
| Accident Procedure                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Report immediately to Dr. Yiming Xiao and/or Dr. Josh Featherstone. Follow all posted procedures in the laboratory.                                                                                                                                                                                                              |
| Emergency Shutdown Procedure                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | In cases where the instrument behaves or sounds abnormal, report immediately to Dr. Yiming Xiao and/or Dr. Josh Featherstone. If not available, turn off all liquid and gas flows then put the instrument in STANDBY from the Tune page. It is not recommended that users turn the instrument off as this can cause more damage. |
| Pre-start Checklist                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                  |
| <ul style="list-style-type: none"> <li>You can book instrument time by emailing YX or JF and requesting a time slot. You can view instrument schedules on the UWMSF website:<br/><a href="https://uwaterloo.ca/mass-spectrometry-facility/instrument-schedule">https://uwaterloo.ca/mass-spectrometry-facility/instrument-schedule</a></li> <li>Instrument tuning and calibration can only be performed by authorized trainers (YX or JF) and should only be attempted by users after extensive training.</li> <li>Check status light on the tune page of the QToF, it should be yellow for Standby. The high vacuum should be lower than <math>5 \times 10^{-7}</math> mbar with no gases on, if not find YX or JF.</li> <li>A tune file should be loaded in the Tune window (eg an .ipr file) – this tune file name will change over time depending on the method you're running</li> <li>If any of these conditions are not met, stop what you are doing and find either YX or JF.</li> <li>You must have a sample introduction method, instrument method, ionization mode and polarity that you want to use.</li> </ul> |                                                                                                                                                                                                                                                                                                                                  |

There are 2 main modes of sample introduction for the Waters QToF:

1. UPLC chromatographic separation of a complex mixture. In this case you need a UPLC column (with a guard column where appropriate) along with a suitable mobile phase and gradient. If you don't have a specific method, one will need to be developed.
2. Direct Infusion: Samples can be introduced directly into the system using either a syringe or an autosampler.

Syringe Introduction: For optimal results, prepare the sample at a concentration of 10 pmol/ $\mu$ L (10  $\mu$ M). As a general guideline, a concentration of 50–100 ng/mL is recommended for small molecules.

# University of Waterloo Mass Spectrometry Facility (UWMSF)

## Waters Synapt G2Si HDMS Standard Operating Procedure (SOP)

Autosampler Introduction: When using an autosampler, a higher sample concentration is required to account for dilution during the process. A working concentration of approximately 1 µg/mL is recommended for small molecules.

Solvents and additives used for mass spectrometry should be HPLC grade at a minimum but **LCMS grade solvents and additives would be the best choice.**

### Start-up, Operating and Shut-down procedure for LC/MS/(MS)

- The status light on the Tune page should be yellow for Standby indicating the instrument is ready for use. The blue status bar should be clearly visible on the front of the instrument – any other color, find YX or JF.
- Before any sample analysis is performed, it is critical to perform a detector setup, mass calibration, lockspray setup and resolution optimization. These must be performed on a daily basis or before a sample list is run. How to perform these steps will be addressed during your training
- Solvents that can be used for reverse phase LC/MS are somewhat restricted but this will be addressed in your training. Commonly used solvents are MeOH, MeCN and H<sub>2</sub>O mixtures with additives such as formic acid and ammonium formate (for +ve ion). Using basic mobile phases can be problematic as some silica-based columns will dissolve under basic conditions.
- Non-polar solvents used for normal phase UPLC require the use of the ESCI option and a different UPLC stack has to be used. Please consult with Facility personnel before attempting.
- Steps necessary to purge and prime the UPLC with MassLynx, install the column, load a different tune file, build a MS method in Masslynx, build and run a sequence and data analysis etc will be covered in your training.
- There are MANY variables in this process and as these are sample and experiment dependent, the exact instrument/experiment setup will be tailored to your requirements during the training that is a requirement before ANY analysis is attempted.
- It is recommended to flush your UPLC column to remove buffers and other mobile phase additives before using, this will be detailed during your training.

### Clean-up

- Remove all your samples and waste from the Facility
- Tidy up lab and bench space

# University of Waterloo Mass Spectrometry Facility (UWMSF)

## Waters Synapt G2Si HDMS Standard Operating Procedure (SOP)

### Maintenance and Repair

- Do not attempt to repair any problems you encounter during your time in the Facility. Typical problems that can arise include plugged sprayer, computer crashes, poor chromatographic performance, poor mass accuracy or precision, vacuum problems etc
- It is important that you are able to determine that there is a problem, again your training will help you with this.
- If any of these or other situations that you don't understand arise, please find/talk with YX or JF

### Appendix

#### 1. Mass calibration process

##### a. Automatic calibration

- i. Inject calibrant through syringe or build-in flow system, a typical flow rate is 10  $\mu\text{L}/\text{min}$
- ii. Go to Intelli Start/normal mode, select Use Calibration Profile, then click Start, and select another already calibrated file, click OK.
- iii. Go to Intelli Start/Configuration Mode, select Create Calibration. Follow the on-screen instructions and choose the mode that requires calibration. If a syringe pump is being used to introduce the calibrant, ensure the "current tune page" is selected for data acquisition. Additionally, selecting the appropriate calibrant is critical, as the calibration wizard requires sufficient signal strength to complete the process successfully.
- iv. Review the calibration report. To remove outliers, right-click the corresponding data points. Once the results are satisfactory, select "Accept Calibration."
- v. ensure the calibration's accuracy, perform a verification process. Acquire mass spec data of the calibrant using the newly established calibration. The resulting data should match (or closely align with) the calibration reference file.

##### b. Manual calibration

Manual calibration is similar to automated calibration; however, the data for calibration is acquired manually. This process is required when a specific mass range needs to be targeted.

- i. Specify the conditions necessary for acquiring data from the reference compound.
- ii. From IntelliStart, click Tune-, and select Acquire > Start.

# University of Waterloo Mass Spectrometry Facility (UWMSF)

## Waters Synapt G2Si HDMS Standard Operating Procedure (SOP)

- iii. Acquire 2 minutes of TOF MS continuum data, with a normal dynamic range for your compound, across the desired mass range. Note: change calibration file to “uncal” before start.
- iv. Process the data ready for calibrating:
  1. From the MassLynx main window, click Chromatogram.
  2. Click File > Open, and open the recently acquired chromatogram.
  3. Click Display > Remove, and remove unwanted chromatograms from the window.
  4. Right-click at the zero end of the trace and drag across the chromatogram to combine 2 minutes of spectra.
  5. Then right click again to display a marker and the spectrum window
  6. In the spectrum window, click Process > Automatic Peak Detection, and click OK to create a centroid spectrum.
  7. From the File menu, click Save Spectrum.
- v. Return to IntelliStart and click Configure > Configuration Mode.
- vi. Click Create Calibration and then Start. Or edit an existing manual calibration profile.
- vii. Click Calibration Profile Editor. Select manual calibration, specify the required m/z and acquisition mode. Note the raw file used for calibration should match the range and mode exactly.
- viii. Browse to the raw data file. In the Select Raw File dialog box, select the file, and click History. Select the saved, centroid spectrum. Click OK.
  1. Result: The Calibration dialog appears.
  2. Check for outliers and remove them from the calibration curve by right-clicking the outlying peak in the reference file spectrum and right-clicking the associated peak on the data file spectrum.
  3. -Accept or reject the calibration.

### 2. General trouble shooting

#### a. Reboot Mass Spectrometry

Rebooting the mass spectrometer is occasionally necessary and is a common step in the troubleshooting process. Communication errors are the most frequent type of malfunction and can often be resolved through a reboot. The rebooting process can be categorized into two types:

# University of Waterloo Mass Spectrometry Facility (UWMSF)

## Waters Synapt G2Si HDMS Standard Operating Procedure (SOP)

- i. **Soft Reboot:** Performed without releasing the vacuum, preserving the system's vacuum state. The Soft Reboot can be operated in two different ways:

1. To reboot the embedded PC (EPC) only:
  - a. In the MassLynx software, close the Tune window.
  - b. On the instrument's rear panel, toggle the EPC Reset switch (the black nob).
  - c. Wait four minutes to allow full reboot.
  - d. Result: The LED strip turns solid white.
  - e. Start the MassLynx software and monitor the instrument console for messages and indications.
2. Electronics Reboot

This procedure will reboot all electrical components except the vacuum module

  - a. Override the vacuum: switch the override switch to "override"
  - b. Flip the Auxiliary/EPC/Electronics switch to off. Note: **do not touch the Vacuum switch.**
  - c. Reboot the operation computer.
  - d. Flip the Auxiliary/EPC/Electronics switch to on. Wait 4 minutes.
  - e. Start Masslynx tune page.

- ii. Ping EPC

The communication between EPC and operation computer can be verified by performing ping EPC.

1. Start command prompt
2. Type "Ping epc"
3. EPC should respond under 1 ms, otherwise the communication between EPC and operation computer is not established.

- iii. **Hard Reboot:** Involves releasing the vacuum and restarting the system from a fully powered-down state. (This situation is relatively rare)

1. Vent: put instrumentation to standby, Click Vacuum > Vent; select Vent Instrument. After approximately five minutes, the turbomolecular pumps slow to half their normal operating speed.

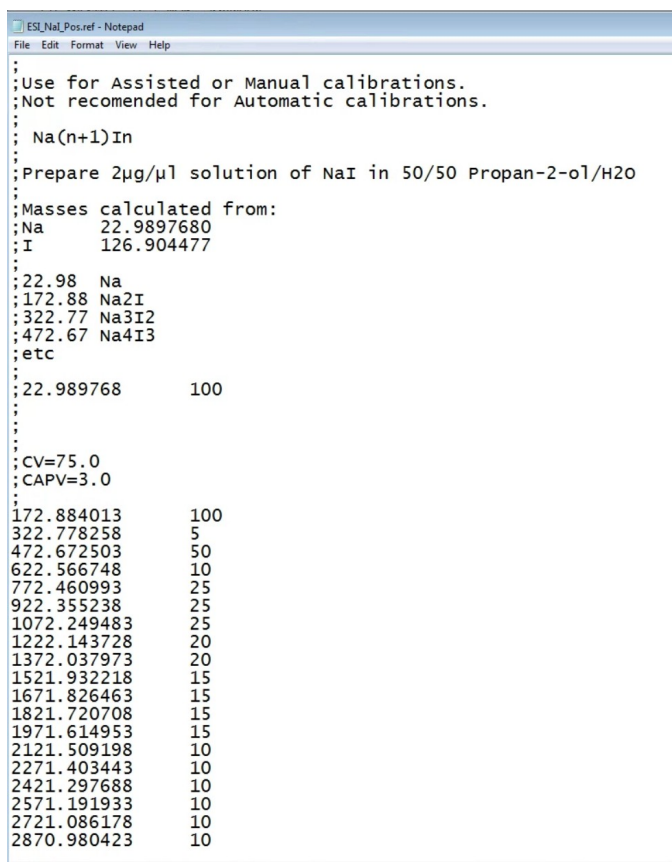
# University of Waterloo Mass Spectrometry Facility (UWMSF)

## Waters Synapt G2Si HDMS Standard Operating Procedure (SOP)

2. Wait until everything stabilizes (as the pump noise frequency reach to the lowest level), Switch off the vacuum, electronics, the embedded PC and auxiliary breakers located on the rear panel.
3. Start: Ensure ACQUITY UPLC is on (The ACQUITY UPLC module that contains the Ethernet switch must be turned on first to allow communications to be established). Then turn on EPC, vacuum, and electronics main power switches.
4. Wait 4 minutes, Click IntelliStart in the MassLynx main window, click operate.
5. Wait at least 2 hours to pump down. If situation allows, let instrumentation fully pump down over night.

### b. Mass reference file location

The mass reference file for each calibration is stored in the "C:\MassLynx\Ref" folder. The acquired MS data for a given calibrate must match the reference file precisely.



```
ESI_NaI_Pos.ref - Notepad
File Edit Format View Help

;
;Use for Assisted or Manual calibrations.
;Not recommended for Automatic calibrations.
;
; Na(n+1)In
;Prepare 2µg/µl solution of NaI in 50/50 Propan-2-ol/H2O
;
;Masses calculated from:
;Na      22.9897680
;I       126.904477
;
;22.98 Na
;172.88 Na2I
;322.77 Na3I2
;472.67 Na4I3
;etc
;
;22.989768      100
;
;
;CV=75.0
;CAPV=3.0
;
;172.884013      100
;322.778258      5
;472.672503      50
;622.566748      10
;772.460993      25
;922.355238      25
;1072.249483     25
;1222.143728     20
;1372.037973     20
;1521.932218     15
;1671.826463     15
;1821.720708     15
;1971.614953     15
;2121.509198     10
;2271.403443     10
;2421.297688     10
;2571.191933     10
;2721.086178     10
;2870.980423     10
```