

# 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.

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, 2020	
SOP revised by	Dr. Richard W. Smith	
SOP Location	C2-268	
User Manual	In LHS cabinet beside the instrument in C2-268	
Equipment owner	UWMSF	
Authorized Trainers	Dr. Richard W. Smith (RWS) and Valerie Goodfellow (VG)	
Authorized Users	Users who have completed the UWMSF Training Certification and all other relevant UW specified safety training	

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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 RWS or VG when deemed necessary by RWS or VG.
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
Relevant MSDS	See chemical inventory binder for relevant MSDS's

Accident Procedure	Report immediately to Dr. Richard Smith and/or Valerie Goodfellow. Follow all posted procedures in the laboratory.
Emergency Shutdown Procedure	In cases where the instrument behaves or sounds abnormal, report immediately to Dr. Richard Smith and/or Valerie Goodfellow. 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 RWS or VG and requesting a time slot. You can view instrument schedules on the UWMSF website: <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 (RWS or VG) 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>5e^{-7}</math> mbar with no gases on, if not find RWS or VG.</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 RWS or VG.</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. APCI GC/MS separation of complex mixtures or purified samples. Setup for this option require the installation of the APGC source and a very different calibration etc process and should only be attempted after extensive training.

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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.**

### 1. 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 RWS or VG.
- 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.

### 2. Start-up, Operating and Shut-down procedure for GC/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 RWS or VG.
- 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 GC/MS are somewhat restricted but this will be addressed in your training. Commonly used volatile solvents are DCM, acetone, ethyl acetate etc.
- Steps necessary to setup the QToF, install the APGC source and attach the Agilent GC and interface, 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.

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- After sample analysis put the instrument in Standby (yellow light on Tune page). The blue status bar should be clearly visible on the front of the instrument – any other color (red, yellow or flashing), find RWS or VG.
- It is recommended to flush your UPLC column to remove buffers and other mobile phase additives before removing and storage again this will be detailed during your training.

### Clean-up

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

### 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 RWS or VG