

University of Waterloo Mass Spectrometry Facility (UWMSF)

Thermo Q-Exactive 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-and-sops>

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.

Name	Thermo Scientific Q-Exactive with Dionex Ultimate 3000 UPLC
Description	Hybrid Quadrupole-Orbitrap LC/MS/MS system with ESI
Location	C2-267
SOP created	December 10 th , 2019
SOP author	Richard W. Smith
SOP revised	Jan 03rd, 2025
SOP revised by	Dr. Yiming Xiao
SOP Location	C2-267
User Manual	In cabinet behind Q-Exactive in C2-267
Equipment owner	UWMSF
Authorized Trainers	Dr. Yiming Xiao (YX) and Dr. Josh Featherstone (JF)
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 YX and/or JF. Follow all posted procedures in the laboratory.
Emergency Shutdown Procedure	In cases where the instrument behaves or sounds abnormal, report immediately to YX and/or JF. If not available, turn off all liquid and gas flows then put the instrument in STANDBY from the Tune page. Close all programs, shutdown the computer, turn off the electronics followed by the main power using the 2 rocker switches on the bottom LHS of the QE. Only perform this operation under extreme conditions! 

Pre-start Checklist

- You can book instrument time by emailing YX or JF and requesting a time slot. You can view instrument schedules on the UWMSF website:
<https://uwaterloo.ca/mass-spectrometry-facility/instrument-schedule>
- Instrument tuning and calibration can only be performed by authorized trainers (YX or JF) and should never be attempted by users
- Check status lights on the top panel of the QE, they should be as follows: 3 green (Power, Vacuum and Status) and 1 yellow (System) indicating that the QE is in standby mode

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- On the tune page, the instrument should be in standby mode (yellow light), the high vacuum should be lower than 2×10^{-10} mbar
- The template tune file should be loaded in the Tune window (eg Pos Neg June 2022) – this tune file name will change over time.
- If any of these conditions are not met, stop what you are doing and find either YX or JF.
- You have a sample introduction method, instrument method, ionization mode and polarity that you want to use.

There are 2 main modes of sample introduction for the Q-Exactive:

1. Infusion ie the direct introduction of a sample without prior chromatographic separation – usually used for pre-purified samples.
2. 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.

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 Infusion

- Before any sample analysis is performed, it is critical to record the chemical noise (background) that is observed for the infusion solvent that you intend to use. This ensures that the previous user hasn't contaminated the system and has cleaned it to the extent that is possible.
- Solvents that can be used for ESI are somewhat restricted but this will be addressed in your training. Commonly used solvents are MeOH, MeCN and H₂O mixtures with additives such as formic acid (for +ve ion) or NH₄OH (for -ve ion)
- Preferred solvents are 1:1 MeOH/H₂O+0.1% formic acid (+ve ion) or 1:1 MeOH/H₂O+0.2%NH₄OH (-ve ion)
- In the Tune window, put the instrument in Operate mode (status light turns green), start the syringe pump (flow rate between 5-10uL/min, never higher than 20uL/min) and wait for the mass spectrum to stabilize. Record the background spectrum.
- You are now ready to analyze your sample – never exceed a concentration of 10pmol/uL (10umolar)
- Fill the syringe, acquire the spectrum and using Xcalibur, analyze your data.
- There are MANY variables in this process and as these are sample and experiment dependent, the exact instrument setup will be tailored to your requirements during the training that is necessary before **ANY** analysis is attempted.

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- When you have finished your analysis, you must again flush the system with the infusion solvent (no sample) and acquire the background mass spectrum showing that the instrument is as clean as it was when you started.
- When the instrument is clean, turn the instrument to Standby (yellow status light) in the Tune window – never turn the instrument to Off (red status light). Record your usage in the Logbook – you have completed your analysis and can leave the instrument for the next user.

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

- Before any sample analysis is performed, it is critical to record the chemical noise (background) Preferred solvents are 1:1 MeOH/H₂O+0.1% formic acid (+ve ion) or 1:1 MeOH/H₂O+0.2%NH₄OH (-ve ion). This ensures that the previous user hasn't contaminated the system and has cleaned it to the extent that is possible.
- 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 H₂O 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 APCI source so a different UPLC stack has to be used. Please consult with Facility personnel before attempting.
- In the Tune window, put the instrument in Operate mode (status light turns green), start the syringe pump (flow rate between 5-10uL/min, never higher than 20uL/min) and wait for the mass spectrum to stabilize. Record the background spectrum of 1:1 MeOH/H₂O+0.1% formic acid.
- Steps necessary to purge and prime the UPLC with Chromeleon, install the column, loading a different tune file, build a MS method in Xcalibur, 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.
- When you have finished your analysis, you must again flush the system with the infusion solvent (no sample) and acquire the background mass spectrum showing that the instrument is as clean as it was when you started. You must load the default Tune file that was active when you came to the Facility, run the infusion solvent that you ran before you started your sample analysis.

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- In the Tune window, put the instrument in Operate mode (status light turns green), start the syringe pump (flow rate between 5-10uL/min, never higher than 20uL/min) and wait for the mass spectrum to stabilize. Record the background spectrum.
- When the instrument is clean, turn the instrument to Standby in the Tune window (yellow status light) – do not turn Off (Red status light). Record your usage in the Logbook – you have completed your analysis and can leave the instrument for the next user.
- The status light on the Tune page should be yellow and the status lights on the QE should be 3 green (Power, Vacuum and Status) and 1 yellow (System)
- 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 YX or JF