

# University of Waterloo Mass Spectrometry Facility (UWMSF)

## Thermo LTQ-XL 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

|                     |                                                                                                                         |
|---------------------|-------------------------------------------------------------------------------------------------------------------------|
| Name                | Thermo Scientific LTQ-XL                                                                                                |
| Description         | Linear Ion Trap LC/MS/MS (and MS <sup>n</sup> ) system with ESI and APCI used for infusion and LC/MS/(MS <sup>n</sup> ) |
| Location            | C2-267                                                                                                                  |
| SOP created         | April 20 <sup>th</sup> , 2024                                                                                           |
| SOP author          | Richard W. Smith                                                                                                        |
| SOP revised         | January 3 <sup>rd</sup> , 2025                                                                                          |
| SOP revised by      | Dr. Yiming Xiao                                                                                                         |
| SOP Location        | C2-267                                                                                                                  |
| User Manual         | On top of the LTQ-XL 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           |



in doubt always ask Facility personnel before trying something outside of your training or prepared protocol.

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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 YX or JF when deemed necessary by YX or JF. |
| 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 Service Mode followed by the Main Power using the 2 rocker switches on the bottom RHS of the LTQ. <b>Only perform this operation under extreme conditions!</b> |

### 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 LTQ, they should be as follows: 3 green (Power, Vacuum and Communications) and 1 yellow (System) indicating that the QE is in standby mode
- On the tune page, the instrument should be in standby mode (yellow light), the high vacuum should be lower than  $1 \times 10^{-5}$  Torr
- The template tune file should be loaded in the Tune window (eg ESI\_Dec17\_2019.LTQTune) – this tune file name will/may 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.

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The LTQ-XL can be used in infusion mode and LC/MS/(MS<sup>n</sup>) modes:

1. Infusion is the direct introduction of a sample without prior chromatographic separation – usually used for pre-purified samples.
2. The Vantage UPLC is controlled from the Xcalibur software and users will be trained to set-up and use this system for their specific project requirements. As each project would have very different requirements, training will be provided to accomplish that project.

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 assess the instrument background by infusing 1:1 MeOH/H<sub>2</sub>O+0.2% formic acid – record this spectrum. 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.
- 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 H<sub>2</sub>O mixtures with additives such as formic acid (for +ve ion) or NH<sub>4</sub>OH (for -ve ion). If you wish to use other solvents or solvent mixtures, please talk with RWS or VG as some solvents can cause damage to the mass spectrometer.
- Preferred solvents are 1:1 MeOH/H<sub>2</sub>O+0.1% formic acid (+ve ion) or 1:1 MeOH/H<sub>2</sub>O+0.2%NH<sub>4</sub>OH (-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.
- When you have finished your analysis, you must again flush the system with the infusion solvent (no sample) and acquire the background mass spectrum.

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- Final step is to record the background spectrum from 1:1 MeOH/H<sub>2</sub>O+0.2% formic acid to make sure the system is as clean as it was before 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<sup>n</sup>)

- The status light on the Tune page should be yellow for Standby indicating the instrument is ready for use – any other color, find YX or JF.
- Before any sample analysis is performed, it is critical to assess the instrument background by infusing 1:1 MeOH/H<sub>2</sub>O+0.2% formic acid – record this spectrum. 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 cannot be used with the Vanquish UPLC as the pump seals are not designed for normal phase solvents. Please consult with Facility personnel before attempting.
- Steps necessary to purge and prime the UPLC with Xcalibur, install the column, load 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.
- 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.
- Final step is to record the background spectrum from 1:1 MeOH/H<sub>2</sub>O+0.2% formic acid to make sure the system is as clean as it was before you started.

### Clean-up

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

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### 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 arise that you don't understand, please find/talk with YX or JF