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PERFORM Operating Document

Use and Maintenance of QQQ LC-MS

PC-POD-CA-001-v03

Revision History

Version	Reason for Revision	Date
01	New POD	11-April-2013
02	Updated to reflect current practices with the installation of the passive nitrogen generator	02-February-2016
03	Minor revision to reflect current procedure for QQQ LC-MS shutdown	22-March-2018

Summary

The content of this PERFORM Operating Document (POD) provides guidelines for the use and maintenance of the QQQ LC-MS.

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1. Definition of Terms and abbreviations

PERFORM operating document (POD)	Operating documents that are specific to an instrument or technique.
LC-MS	Liquid Chromatography-Mass Spectrometry
MS	Mass Spectrometer
QQQ	Triple Quadrupole
Qual	Qualitative Analysis
Quan	Quantitative Analysis
UPLC	Ultra Performance Liquid Chromatography
HPLC	High Performance Liquid Chromatography
N_2	Nitrogen
ESI	Electrospray Ionization
APCI	Atmospheric Pressure Chemical Ionization
BinPump	Binary Pump
TCC	Thermostat Column Compartment
TFA	Trifluoroacetic acid
MP	Mobile Phase
EMV	Electron multiplier voltage
MW	Molecular weight
L	Liter
Min	Minute
µL	Microliter
mL	Milliliter
µA	Micro Ampere

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2. Introduction

The PERFORM Centre Clinical Analysis suite has an Agilent 6460 Triple Quad Mass Spectrometer (LC-MS) which is a bench top QQQ providing atmospheric pressure ionization of liquid samples. A wide variety of compound classes may be analyzed using either ESI or APCI. Molecular weight information as well as structural information through collision induced dissociation provides useful qualitative sample information. This is well suited for both routine and complex analyses such as metabolism, toxicology, environmental, food safety, pharmaceutical, and drugs of abuse testing. This system provides analysis with greater sensitivity and productivity for quantifying pharmaceutical candidates, measuring trace-level environmental or food contaminants, or confirming biomarkers. Mass Hunter Quantitative Analysis software provides quantitative results with minimal manual intervention.

2.1 Overview of LC-MS

- The 1290 Infinity II LC System(UPLC 1290) is equipped with a binary pump for two pairs of solvents (A1,A2 and B1, B2) and is capable to withstand 1200 Bars of operational pressure. Column and sample temperature is controlled by using column and auto-sampler thermostats.
  The chromatography system is coupled to mass spectrometer via ESI or APCI interfaces (sources) which are interchangeable.
- Two supplies of nitrogen are required to run MS analysis; One, from nitrogen tank (ultrapure grade) to operate the collision cell and another from N_2 generator to operate ESI or APCI source, The first source is equipped with filters which should be replaced at least once a year or more frequently if required by workload. The second source of N_2 is supplied by a passive N_2 generator connected to a stationary source of Quebeair. The pressure should be maintained sufficiently high to provide 80-100 psi pressure of nitrogen at the generator output. Diffusion/selection membrane in passive generator should be replaced at least once a year.
- There are 3 pumps (1 mechanical and 2 turbo) to create the vacuum in LC-MS system.
- There are 2 ion sources- APCI and high efficiency Jet stream ion source ESI which are readily interchangeable.
- Built-in system logbook keeps log of errors for the sample running. Logbook deletes old entries each time a new worklist is run so it should be either saved or printed if required to report any malfunction of the instrument.
- Mass Hunter software suit is used for data analysis and is suitable for R&D purposes. For compliance, additional software can be purchased.

2.2 Training requirements

Prior to using the LC-MS System individuals should:

- Read and sign this POD.
- Undergo appropriate LC-MS System training and/or provide a proof of an external training to the Supervisor, Clinical Analysis prior to use.

2.3 Booking and operation

Reserve the LC-MS system using online scheduler available at: http://perform.concordia.ca/booking

In order to access the PERFORM Centre online Booking system for facilities and equipment, an account on the system will be required. Accounts are only created for people who are approved to do research at the PERFORM Centre. The Principal Investigator can request access and accounts for themselves and their research assistants online at:
https://perform.concordia.ca/GettingStarted/forms.asp

The Principal Investigator (PI) can also request a PDF version or paper copy of the account request form from the PERFORM Centre's Research Operations Coordinator or System's Administrator.

2.4 General precautions

- Use only water and high purity solvents (LC-MS grade): do not use water generated by Millipore system in the lab. Avoid contamination by using clean pipets, bottles and glassware.
- Make sure the level of oil in the mist filter from the mechanical pump is cleared regularly by opening the ballast valve for a few minutes. MS must be on standby before the ballast valve is opened. The valve should be closed for the routine operation. Make sure that there is no leak of oil. Check the oil level in the rough pump.
- If the rough pump overheats, it will beep. Let it cool by opening the front cover.
- The oil in the rough pump should be replaced and MS ion transfer capillary cleaned every 6 months. About 1.5 L of oil (Agilent 6040-0834) is required for replacement. To change the oil, turn off the LC-MS and shutdown the MS: In order and from the software, UPLC is on standby mode, right click on QQQ pane in the Data Acquisition software and press Vent. Wait 40 minutes. Then close the valve from the compress air (on the wall 90° clockwise). Turn off the computer, switch off the MS, UPLC, and the oil pump via their power buttons. The oil pump and capillary can be cleaned. To turn on the MS, open the compress air slowly and then manually turn on UPLC, MS and oil pump. Wait until UPLC injector finish the initialization. Turn on the computer, open Data acquisition software, right-click on Pump Down in the MS pane. Wait minimum 4 hours before using the MS and proceed to an

autotune before any run.

- Verify the pressure from the Nitrogen generator, outlet pressure should not be below 80 psi, pressure is directly correlated with the sensitivity of the system.
- Check the calibrant level before tuning. Use different tuning mixes for both ESI and APCI sources available in the lab.
- Verify that all the cables are properly secured.
- It is important to use appropriate fittings with columns, valves and unions. Agilent Technologies uses Swagelok fittings but for Waters columns use Waters fittings.
- Agilent Capillary tubing is color coded to identify the internal diameter e.g. Red= 0.12 mm, Green= 0.17 mm and Blue = 0.25 mm. Make sure to use the right tubing and fittings for the connections.
- The binary pumps should be purged and primed before use.
- Drain any liquid from mass spec waste bottle otherwise it will flush back to LC-MS.
- Set the pressure limits according to your column.
  Buffers should be <15 mM and filtered prior to use. Never leave pure acetonitrile on the system as it will polymerize if left standing for periods of time. Always remove the column and store the system with 10% acetonitrile in HPLC grade water.
- MP bottles should never be covered with Para film. Use Aluminum foil. It is recommended to use amber bottles for the Water on the system.
- If the sample injection is required using a 96 well plates, configure the software for 96 well plates. By default it is configured for injection using vials.
- Flush the system with water right after if the buffer has been used to get rid of the salts. When changing solvents, all channels should be purged at 2 mL/min for 2 minutes/channel.
- If the column is not in use, rinse it with additive free mobile phase and then with 90-100% acetonitrile at 0.1 mL /min for 10-20 column volumes or as recommended by the column manufacturer.
- If very sticky compounds are used, extra cleaning is required to remove traces. An Injector Purge Kit is available from Agilent Technologies for extra cleaning for sticky compounds.
- Triple Quad has 0.5 micron on-line filter installed after injector and before column compartment which should be replaced after some time or upon rising HPLC pressure for more than 5%.
- To change the APCI source put system on standby. Yellow light "should turn on" on standby mode. Wear gloves while replacing the regular spray shield for APCI because it may be hot.
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2.5 Relevant documents

- Agilent 6460 Series LC-MS QQQ techniques and operation **volume I** Student Manual

- Agilent 6460 Series LC-MS QQQ techniques and operation **volume 2** Student Manual
- Nitrogen generator Manual

3. Procedure

3.1 MS Operation

If the system is not turned on, follow the sequence below to turn it on:

- Check the pressure in N_2 generator first for the pressure above 80 psi
- N_2 cylinder is always open for collision energy
- Turn on the triple Quad
- Turn on the UPLC and wait for 5 minutes.
- Login with the given user name and password.
- Go to the Mass Hunter Data Acquisition application and turn on the system.

Always perform auto tune once the system is turned "ON".

If the mass spectrometer is not in use, keep in standby mode. Never turn the system off except for appropriate maintenance or cleaning.

Follow the instructions from page 195-204 of the student manual LC-MS QQQ techniques and operation volume 1 for preparing UPLC and sample preparation procedure. A QQQ routine pre-operation checklist is included in Appendix I and can be used to verify the LC-MS operation.

If any component of LC-MS system does not work inform the Supervisor, Clinical Analysis or delegate. Fill out a malfunction form available in APPENDIX III of this POD if the malfunction is minor e.g. due to power failure etc. and the system is fixed by taking minor actions. If the system is damaged do not attempt to fix it and fill out a damaged or lost equipment form available in PC-SOP-GA-004, Asset management at PERFORM and inform Supervisor, Clinical Analysis.

3.2 Data acquisition software layout and instrument controls

Open the data acquisition software. There is a default layout which can be used.
The status of the instrument can be determined by looking at the instrument status pane. Green means that the instrument is ready for a run. Yellow means "not ready", red means that an error has occurred in the module and you can check the logbook to view the error or hover over the red bar for a disruption of the error. Blue means that the system is running and a pink color indicates that the instrument is waiting to inject.

Turn BinPump on and flush the injector. Ensure that lines are clear of bubbles, if not

purge the lines via the BinPump pane. Flush the mobile phase from the binary pump for 60 sec. In TCC (thermostat column compartment), with direct injection, switch to column 1 or 2 depending on the choice of the column. Under "Bin Pump" icon you can set mobile phase composition, gradient steps and times, flow rate. Turn the pump on.

Save the data in R: drive with your folder. Contact PERFORM IT analyst in order to obtain access to this drive.

Refer to pages 365-418 of the student manual LC-MS QQQ techniques and operation volume 1 to set up all UPLC and QQQ parameters.

Follow the procedure below if you need to perform a Checktune or Autotune on LC-MS.

3.3 Check Tune and Auto Tune

Refer to page 171 of **student manual LC-MS QQQ techniques and operation volume 1 for Checktune and Autotune procedure.**

- Wait at least 15 min to stabilize before tuning. There are different low concentrations tuning mix solutions for ESI and APCI sources from Agilent Technologies. The flow rate for tuning mix is 0.3 mL /min. Make sure that there is enough calibrant in the bottle for Checktune or Autotune.
- Check tune once a week when in use or as specified in the study protocol. If one or two masses do not pass then do Autotune.
- Autotune will automatically change the QQQ parameters and calibrate the QQQ. Routine use only requires resolution and mass axis verification for polarity and resolution modes to be used. Select the ions of interest as Positive or Negative for the Check tune. It will take 15-20 minutes for every mode. No need to inject anything as it is automatically run by the system. If there is no peak in the Autotune, a number of issues may be causing this: the source is dirty, ion transfer capillary is blocked, there's no calibrant solution left, and/or calibrant solution is not being delivered to the source. This is not an extensive list but can help to start troubleshooting. To clean source flush it with 50:50 methanol and HPLC grade water before repeating.
- After Autotune the EMV is usually 2000 V for old instrument. For the new instrument the EMV is around 1100 V.
- Check the results if they are acceptable or not. The report will be automatically saved as PDF under D:\Masshunter\tune\QQQ\G6460A.
- Autotune should be done at least quarterly to optimize ion transmission and update EM voltage. It takes about 60 minutes to run the Autotune for both Positive and Negative modes. Flush source with LC flow after Autotune before running samples in negative ions. If using negative ion, Autotune should be done monthly or less to minimize source exposure to TFA.

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If Checktune fails and abundances drop dramatically, in parallel with a major loss in assay sensitivity, this suggests a problem with the source and/or mass analyzer. A major drop in tuning mix mass without a loss in analyte signal suggests a calibrant problem. The calibrant level may be low or it has been degraded or the diverter valve may be malfunctioning. Inform Supervisor, Clinical Analysis if it happens, who will contact service provider.

Note: It is useful to monitor the Autotune EMV in the Autotune report in order to know when the electron multiplier will need to be replaced. When the sum of the Autotune EMV and the method delta EMV approach 3000 volts, a new electron multiplier tube should be replaced after venting the LC-MS system. Inform Supervisor, Clinical Analysis who will place a service call.

3.4 Work list

Refer to pages 5-27 of the training manual (volume 2) for the work list preparation.

3.5 Qualitative analysis

Refer to pages 52-104 of the training manual (volume 2) for the Qualitative data analysis preparation.

3.6 Quantitative Analysis

Refer to pages 178-240 of the training manual (volume 2) for the Qualitative data analysis preparation.

3.7 Parameters to use in the method optimization

For the method optimization, the parameters for Jet stream ESI and APCI are shown in Appendix II of this POD.

3.8 Preventive maintenance

Follow **Agilent 6400 Maintenance Guide** for detailed procedures. In general the following is required for the regular maintenance of LC-MS when in use:

Daily:
Flush the nebulizer, clean electrospray spray chamber, clean the APCI chamber, clean multimode source, check drain bottle.

Weekly:
Clean electrospray spray chamber, clean the APCI chamber, Clean multimode source, and check the level and color of the roughage pump fluid. Check the oil mist filter

Monthly:
Flush the nebulizer. Replace the pump oil every six months. Replace it sooner if the oil appears dark or cloudy.

Annually:
Replace MS selection wall rotor seal. Multiplier needs replacement every year. Change Hydrocarbon filters. Wipe the skimmer once a year.

When required:
Clean and adjust electrospray needle as well as the APCI needle. Clean the capillary, clean skimmer, change lamp in the source, check for leaks, replace the LC filter elements, replace electron multiplier horn, replace high energy dynode. Add rough pump fluid when the pump fluid level is low. Replace the fuses. Change the seal of the pump when required, needle wash and needle seat after 60 000 injections

To adjust the needle, put it in to the magnifier and adjust the needle so that it is aligned with the tip of ESI or APCI. If it is blocked, dip it in 50:50 methanol and Type 1 water and sonicate it for 3 minutes.

Nitrogen generator:
Refer to Nitrogen generator Manual for the operation, troubleshooting and maintenance details. Below is the maintenance schedule for N_2 generator:

Part	Action	Frequency
Filters	Replace carbon absorber	1x per year
Oxygen sensor	Replace oxygen sensor	1x per 3 years
Oxygen sensor	Calibrate oxygen sensor	1x per year
Air compressor	Replace on failure	Expected life > 8000 hrs
Nitrogen compressor	Replace on failure	Expected life > 8000 hrs
Battery on PCB	Replace on failure	Expected life > 5 years

APPENDIX I

QQQ Routine Pre-operation Inspection Checklist

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QQQ Routine Pre-operation Inspection Checklist

System Components	Items			
Degasser	Check status			
Rough Pump	Clear demister			
	Check oil level			
	Rough vacuum			
Turbo Pump	High Vacuum			
Nitrogen Generator	Pressure			
Collision Cell	Nitrogen (psi)			
Source	Cleaning (if required)			
	Check Nebulizer spray			
	Inspect Nebulizer tip			
	Chamber current (ESI or MMI)>0.25µA			
	Capillary current (ESI or MMI)> 20 nA			
Mobile phase/Pump	Prepare Mobile Phases			
	Set solvent levels			
	Check/empty waste			
	Prime pump			
	Column pressure			
Autosampler	Flush pump solvent level			
	Flush pump prime			
	Reset			
Tuning	Check calibrant level			
	New calibrant (if low)			
	Checktune			
	Autotune (if checktune fails)			
MS2 Scan	Background Check			
System	Suitability test mix			
	Carryover Check			
Initials	N/A			
Date	N/A			

APPENDIX II

Start-up parameters for QQQ LC-MS (small molecules)

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Start-up parameters for QQQ LC-MS

Typical starting parameters for Jet stream ESI and APCI are shown below:

Parameter	Typical starting values for Jet stream ESI (small molecules)	Typical starting values for APCI (small molecules)	Increment and ranges to test	Things to note
Sheath gas temperature	250-400 °C	N/AP	50°C, 250-400°C	Requires time to stabilize
Sheath flow	11-12 L/min	N/AP	2 L/min, 8-12 L/min	Generally 10-12 L/min for 0.2-0.7 mL
Drying Gas temperature	300 °C	350°C	50°C, 250-350°C	Interaction with flow and sheath parameters
Drying Gas flow	5-7 L/min	5 L/min	2L/min, 5-11 L/min	Generally 5-7 L/min, higher when in doubt
Nozzle voltage	500 V (at 0.25-0.6 mL/min LC flow)	N/AP	500V, 0-2000V	Very compound and polarity dependent
Nebulizer pressure	45 psi	60 psi	5 psi, 25-50 psi	LC flow and mobile phase dependent
Capillary Voltage	4000V (Positive), 3500 V (Negative)	3500 V	500V, 2500-4500V	Somewhat MW dependent
Corona Current	N/AP	4 µA (positive), 25 µA (negative)	0.1,0-10 µA (Positive), 1, 0-100 µA (Negative)	Very compound dependent
Vaporizer temperature	N/AP	350°C	0-500 °C	LC flow dependent

Note: Fragmentor V and Collision Energy (for MS/MS systems) should be optimized first using recommended starting parameters for source.
Voltages, gas flows change quickly and can be optimized with series of flow injections using method with injector program and time segments.
Temperatures (sheath gas, drying gas, and vaporizers) require equilibration time between injections; perhaps best done with final chromatography conditions.

Reference: Agilent 6400 Series Quadrupole QQQ Method Development Triple and Optimization LC-MS/MS Users Session by David Presser, Application Scientist

APPENDIX III

QQQ Malfunction Form

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QQQ Malfunction Form

User Information			Details of malfunction	Corrective action taken	Date of Checktune /Autotune performed and passed (dd/mm/yyyy)
Name	Department	Ext.			

APPENDIX IV

POD Training Record Form

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Use and Maintenance of QQQ LC-MS

Ownership	Document type	Area	SOP Number	Version
PC	POD	CA	001	V03

Training Record

Full Name	
Institution	
Contact (email or phone number)	

Signature

Sign here

Date