

PERFORM Operating Document

Use and Maintenance of WATERS Ultra Performance Liquid Chromatography (UPLC) System

PC-POD-CA-003-v03

Revision History

Version	Reason for Revision	Date
01	New POD	10-Jun-13
02	Revised POD. Minor revisions of section 2.5.	20-April-2016
03	Revision of sections 2 and 3.	24-May-2018

Summary

The content of this PERFORM Operating Document (POD) provides guidelines for the use and maintenance of the Waters UPLC.

Table of Contents

SUMMARY	1
1. DEFINITION OF TERMS AND ABBREVIATIONS.....	3
2. INTRODUCTION.....	3
2.1 Overview of UPLC.....	4
2.2 Training requirements.....	4
2.3 Booking and operation.....	4
2.4 General precautions	5
2.5 Relevant documents	6
3. PROCEDURE.....	6
3.1 UPLC Operation	6
3.1.1 General Operating Practices.....	6
3.1.2 Daily Start-up.....	6
3.1.3 Recommended solvent list for UPLC:.....	7
3.2 Data acquisition software layout and instrument controls.....	8
3.3 Run Samples	8
3.4 Processing method.....	8
3.5 Preventive maintenance.....	8
APPENDIX I: POD Training Record Form	

I. Definition of Terms and abbreviations

PERFORM operating document (POD)	Operating documents that are specific to an instrument or technique
UPLC	Ultra Performance Liquid Chromatography
HPLC	High Performance Liquid Chromatography
BinPump	Binary Pump
TCC	Thermostat Column Compartment
MP	Mobile Phase
MW	Molecular weight
L	Liter
Min	Minute
mcL	Microliter
mL	Milliliter
µA	Micro Ampere
TFA	Tri fluoro acetic Acid
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
TEA	Tri Ethyl Amine
EDTA	Ethylene Diamine Tetra Acetic acid

2. Introduction

PERFORM Centre Clinical Analysis suite has a Waters UPLC system with a Photodiode Array (PDA) detector. The ACQUITY UPLC® provides significantly more resolution while reducing run times, and improves sensitivity for the analyses of many compound types.

The PDA Detector provides detection and quantification of very low concentrations of sample analytes in a very short time and compares spectra across wavelengths and broad concentration ranges. It can be used for the identification of components that are difficult to resolve by conventional HPLC based methods.

PERFORM Centre

Features include:

- Quantitates trace impurities at levels to 0.004% with low signal-to-noise ratios, high optical and digital resolution
- Achieves separations with flexible parameter control and sampling rates for accurate, reproducible integration
- Offers efficient light transmission for maximum sensitivity
- Provides simultaneous 2D and 3D operation in Empower™ Software
- Wavelengths of 190 to 500 nm and up to 2.0 AU without compromising linearity

2.1 Overview of UPLC

The ACQUITY H Class System allows scientists to move up to UPLC while making it easier to run existing HPLC methods on the same instrument. The system consists of the following:

-Quaternary Solvent Manager (QSM)

- Capable of running up to four Solvents low pressure gradient mixing
- Supports UPLC pressure
- Low gradient delay volume

-Sample Manager-Flow Through Needle (SM-FTN)

- One injection mode-direct inject type
- Much Simpler-Just specify injection volume
- Excellent recovery and precision
- Single wash station to wash the needle both the inside and the outside

-HTCH-A

- High temperature column heater with active pre-heating
- The temperature range for operation is 5°C above ambient to 90°C.

-PDA Detector

- It allows the laboratory to detect and quantify lower concentrations of sample analytes and compare spectra across wavelengths and broad concentration ranges.
- It has data rates of up to 80 Hz, noise specifications of $\pm 3 \mu\text{AU}$, and an extended linear range.

2.2 Training requirements

Prior to using the UPLC System individuals should:

- Read and sign this POD.
- Undergo appropriate UPLC System training and/or provide a proof of an external training to the clinical analysis supervisor prior to use.

2.3 Booking and operation

Reserve the UPLC system using online scheduler available at:
<http://perform.concordia.ca/booking>

In order to access the PERFORM Centre online Booking system for facilities and

PERFORM Centre

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 HPLC grade water or type I water from the Millipore water system in the lab. Avoid contamination by using clean pipets, bottles and glassware. Always use high purity solvents (HPLC grade).
- Verify that all the cables are properly secured.
- It is important to use appropriate fittings with columns, valves and unions. For Waters columns use Waters fittings.
- Set the pressure limits according to your column. The upper limit of the flow rate is 2 mL/min.
- 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 store with 10% acetonitrile in HPLC grade water.
- MP bottles should never be covered with Para film because it dissolves. Use Aluminum foil.
- 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 48 vials.
- Initialization: Open uHPLC module one by one by pressing button "on" from the the pump and the autosampler. Each part will beep three times and runs series of initialization lasting about seven minutes. Do not turn on the detector. Flush the system with HPLC grade methanol or acetonitrile for at least 15 minutes to ensure detector cell is filled with solvent and free of bubbles. Solvent must be sonicate for 5 minutes, even if the system is equipped with an integrate degasser. Then turn on detector, The LED green light will be flashing while the detector is performing test, it will show steady green when the lamp is ignited. After internal calibration of each module are done then software can be opened; power on acquity and start

PERFORM Centre

Empower (see section 3.1.2 below).

Flush the system right after if the buffer has been used to get rid of the salts. When changing solvents, all channels should be purged at 2500 mL/min for 2 minutes.

- If the column is not in use, rinse it with buffer free mobile phase and then with 90-100% acetonitrile at 0.2 mL /min for 10-20 column volume. To condition the column, in Empower, open run samples and select a separation method that includes the chromatographic conditions, in the samples table add a row to the method. Select equilibrate/condition column (isocratic or gradient) as the function in the new row. Run the separation method. The system runs the gradient.
- If very sticky compounds are used, extra cleaning is required to remove traces.

2.5 Relevant documents

- UPLC Operators manual
- Waters Corporation registered 2010. ACQUITY H – Class for Lab Practitioners
- EMPOWER Software Getting Started Guide

3. Procedure

3.1 UPLC Operation

3.1.1 General Operating Practices

- Prepare fresh solvents daily. DO NOT top-up aqueous solvents.
- Use a fresh reservoir bottle to avoid bacterial contamination.
- Make up only 500 mL or less at a time to minimize the waste.

3.1.2 Daily Start-up

Turn the system on by pressing buttons on pump and autosampler, and then detector as mention in section 2.4.

There are two ways to access the UPLC console:

- In Empower, use Run Samples window, browse project, PERFORM, use quick start,ok
- Use the Desktop Stand-alone ACQUITY Icon
- Prime solvents for the solvent manager when the system is idle for few hours or changing reservoir /solvents. Go to QSM and then Control > Prime Solvents to prime all 4 solvent lines with mobile phase for 5.0 minutes each. For priming the seal wash go to Control > Prime seal wash and select “Yes”. To stop click

PERFORM Centre

- Control > Prime Seal wash. The priming flow rate for QSM is 4.0 mL/min therefore ensure that the solvent reservoir contains enough solvent for adequate priming.
- Prime wash solvent for 15 sec and purge solvent for 5 cycles by selecting FTN sample manager, Control > Prime. To wash needle go to Control > Wash needle for 6 sec.
 - Equilibrate the column temperature by turning the column heater on and setting up the temperature. The column compartment is equipped with an e Cord technology which keeps the paperless column history, certificate of analysis, QC test data and column usage data.
 - Turn on the lamp 30 minutes prior to warm up.

These operations can be done simultaneously.

3.1.3 Recommended solvent list for UPLC*:

Recommended Solvents Methanol, water, Acetonitrile, Isopropanol
Sample diluents (in addition to the solvents listed above) DMSO and DMF
Additives 0.2% Formic Acid, 0.1% TEA, 10mM Phosphate buffer, 50 mM ammonium hydroxide, 0.1% EDTA, 0.1% TFA, 50 mM ammonium acetate, 10mM ammonium bicarbonate
Non-Recommended Solvents Methylene Chloride, Chloroform, Strong acids >5%, Ethyl Acetate, Toluene, Chlorinated Solvents (Trichlorobenzene)

*Waters Corporation registered, 2010. ACQUITY H - Class for Lab Practitioners.

Note: Never use nitric acid to wash the UPLC system as it will destroy the system components.

Detector must be shut down when not in use e.g. in between the analyses, overnight, for a weekend etc. Before turning it off make sure all the buffer has been washed away from the detector.

If any component of UPLC system does not work inform the clinical analysis supervisor or designate. If the malfunction is minor e.g. due to power failure etc. and the system is fixed by taking minor actions continue the work. If the system is damaged, do not attempt to fix it and fill out a lost or stolen report form available in PC-SOP-GA-004, Asset management at PERFORM and inform clinical analysis supervisor.

PERFORM Centre

3.2 *Data acquisition software layout and instrument controls*

Empower Software provides four different user interfaces:

- QuickStart
- Pro
- Open Access
- Web

3.3 *Run Samples*

Follow EMPOWER Software Getting Started Guide for the detailed procedure.

First turn on the UPLC and wait a moment before booting up the Empower program as mention in section 2.4. To log in enter the Username and the password and then choose Advanced and then Quick start. Set up the parameters for your method and save it. Define plates/vials accordingly. Select location of the standards. Enter the information of the samples and run samples. Set up your mobile phase conditions and flow rate using the Quaternary Solvent Manager (QSM) before starting the batch. For needle specification, use sample manager ACQ-FTN, advanced, needle placement is by default at 4 mm, for 48 UPLC vials, vials 12 x 32 mm. If you highlight Manager FTN, configure, volume, sample syringe size is 15 ul by default. Meanwhile no more than 10 µl should be injected, if more is needed, a sample loop could be added and the setting must be changed through the software.

3.4 *Calibration and Quality control*

Follow acceptable methods of calibration with pure standards to calibrate methods. Use a minimum of five standards to generate a standard curve. The concentration range should cover the entire range of quality-control samples. It is recommend to routinely run three quality-control samples. Quality-control samples should represent subnormal, normal, and above-normal levels of a compound, sample results have to be in acceptable range and evaluate precision from day to day, and run to run. Data collected when quality-control samples are out of range may not be valid.

3.5 *Processing method*

Follow EMPOWER Software Getting Started Guide for the detailed procedure.

From the Quick start interface, select the "Browse Projects" tab. From there you will see your latest run. Right click on the desired sample set and click on "Process". Create a processing method and apply it. Review your results and generate a report.

3.6 *Preventive maintenance*

Follow **Waters UPLC operator's manual** for detailed procedures. In general the following is required for the regular maintenance of UPLC when in use:

PERFORM Centre

Annually:

- 3.6.1** Replace the check valve, solvent manager plungers and head seals on the binary solvent manager
- 3.6.2** Replace the sample needle assembly, sample loop, sample syringe and wash syringes and injection valve cartridge on the sample manager
- 3.6.3** Replace the lamp on the UV detector
- 3.6.4** Clean the air filter in the sample organizer

During Preventive maintenance:

- 3.6.5** Replace the mixer on the binary solvent manager
- 3.6.6** Replace solvent filters. Replace the air filters in the binary solvent manager
- 3.6.7** Replace the filter in column in line filter unit on the column heater

As needed:

- 3.6.8** Replace the vent valve cartridge, defrost the sample compartment
- 3.6.9** Whenever the needle is replaced, calibrate the needle z-axis on the sample manager; characterize the needle seal, needle and the sample loop volume.
- 3.6.10** Replace the puncture needle holder on the sample manager
- 3.6.11** Modify the sample needle configuration
- 3.6.12** Test the back pressure regulator
- 3.6.13** Purge the flow cell, reverse flush the flow cell, and replace the flow cell.
- 3.6.14** Replace the fuses
- 3.6.15** Clean the system instruments with a soft cloth

APPENDIX I

POD Training Record Form

PERFORM Centre

Use and Maintenance of Waters UPLC System

Clear Form

Ownership	Document type	Area	SOP Number	Version
PC	POD	CA	003	V03

Training Record

Full Name	
Institution	
Contact (e-mail or phone number)	

Signature

Sign here

Date