

## STANDARD OPERATING PROCEDURE

[High Performance Liquid Chromatography]

Doc No. HPLC-  
Vanquish-1.0

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**Instrument:** HPLC

**Brand/Model:** ThermoFisher Vanquish

**Software:** Chromeleon 7

### 1 INTRODUCTION

High Performance (or Pressure) Liquid Chromatography (HPLC) is an analytical technique that separates the components of a sample and detects them using various detectors — most commonly UV, RI or CAD — each suited to different compound types. The choice of detector depends on the optical and chemical properties of the target analyte.

### 2 SCOPE

The scope of this standard operating procedure (SOP) outlines the safe use and operation of the ThermoFisher Vanquish HPLC by following an established method. The SOP will describe the operation and analysis utilizing the Chromeleon 7 software to control the HPLC.

This is considered a Low-risk item.

### 3 REQUIREMENTS

#### 3.1 Roles and responsibilities

**Instructor** is a TrACEES Platform staff member who has adequate expertise and training responsibility for the instrument.

**Instrument supervisor** is the Platform staff who is responsible for maintenance and troubleshooting.

**Operators (or Users)** are any internal (incl. the University's students and staff) and external personnel.

#### 3.2 Personal Protective Equipment (PPE)

Laboratory coat, safety glasses, fully covered footwear, and gloves when working in the laboratory.

#### 3.3 Training and Induction

- Refer to the HPLC workflow document (PowerPoint format) located on the desktop for detailed procedural guidance.
- Prerequisites TrainMe Modules: PPE, Laboratory Safety and Chemical Management.
- All users shall complete a local lab induction and be trained by the instructor.
- Users from Schools/Departments other than the School of Chemistry shall also complete a building induction.

### 4 WARNINGS

#### Hazards

- Connecting HPLC column while pump is running may cause pressurized acidic eluent to leak, leading to skin exposure.
- Ergonomic strain from lifting bottle above head height; risk of spillage and eye contact during connection.
- High system backpressure if waste valve remains closed during purging.
- Leakage from column connections after starting pump due to loose fittings.
- Hand injury from interacting with moving autosampler rack/ injection needle.
- Disconnecting HPLC column while pump is running may cause pressurised acidic eluent to leak, leading to skin exposure.
- Handling of glass sample vials

### 5 OPERATION

#### 5.1 Start Up

##### 5.1.1 Start-up

- Ensure the intended instrument configuration (UV/RI or CAD) has been set up prior to use. Refer to the HPLC PowerPoint workflow (**Slides 6 and 47 - 48**). This is a **critical step** to confirm that the flow path is directed to the correct detectors and to prevent mixing of salt-based aqueous eluent (for UV/RI detection) with

organic solvent (for CAD detection), which can cause precipitation and instrument damage.

- Double-click the following icon to open the Chromeleon 7 software.



### 5.1.2 Flushing

#### *UV/RI configuration*

- Connect your eluent bottle to the desired solvent line. You can choose A1, A2, B1 and/or B2 for UV/RI detection.
- Connect your column.
- Navigate to Vanquish Pump and enter the information about eluent line selection and %eluent.
- Set the flow rate to 1 mL/min to replace the MQ water in the line with your solvent. Turn on the flow and allow it to continue for around 1 hour.
- Click Monitor Baseline.
- Monitor for backpressure and any leakage.
- If UV/Vis detection is required, turn on the lamp(s) to allow lamp housing to warm up and stabilize before analysis. This warm-up phase will take around 1 hour. The lamps can only be turned on once a flow has been established through the system.
- If RI detection is required, navigate to the RI detector page and select Purge to prepare the detector.
- Wait for the pressure and UV/RI baseline to become stable before commencing analysis.

#### *CAD configuration*

- Connect 40% acetonitrile to line A3 and/or B3.
- Connect a union fitting in the column compartment if your column is not compatible with 40% acetonitrile.
- Ensure that the flow path has been directed to the CAD detector (not to the UV/RI detector).
- Turn on the nitrogen gas supply to the system and ensure 'Gas State' is turned on.
- Navigate to Vanquish Pump. Set the %eluent accordingly.
  - If using one eluent line, set the eluent percentage to 100%. Set the flow rate to 1 mL/min.
  - If using both lines, set A3 and B3 to 50% each. Set the flow rate to 2 mL/min.
- Click Monitor Baseline.
- Flush the lineup for 1 hour.

- Monitor system backpressure and CAD baseline throughout.

### 5.1.3 Purging

- Ensure your eluent bottle is connected to the desired solvent line. You can choose A1, A2, B1 or B2 for UV/RI detection; A3 or B3 for CAD detection.
- Ensure column is connected.
- Navigate to Vanquish Pump and enter the information about eluent line selection and %eluent.
- Open the waste valve by 1.5 turns counterclockwise.
- Click Purge. By default, the process takes 60 seconds.
- Once purging is complete, close the waste valve.

## 5.2 During Operation

### 5.2.1 Create Sequence

- Navigate to your user folder.
- Select the 'Create' in the top ribbon and select 'Sequence'.
- Follow the prompts of the Sequence Wizard. Refer to the HPLC PowerPoint workflow document for guidelines.
- Load your samples onto the autosampler.

### 5.2.2 Load or Create Instrument Method

- Load an instrument method onto your sequence or create a new instrument method using the Instrument Method Wizard (refer to the steps outlined in the HPLC PowerPoint workflow).
- Review your instrument method in the Chromeleon Chromatography Studio. Here, you can verify and adjust all method parameters, including pump settings, autosampler settings, column temperature, and detector settings.
- **IMPORTANT: Review the Shutdown/Standby settings to ensure the system behaves as intended after runs.**

### 5.2.3 Start HPLC analysis

- Ensure all information required in the sequence has been populated.
- At the top of the sequence list, click 'Add to Queue'.
- Go to Options and ensure that either Smart Standby (for short breaks, e.g., ~1 hour before the next run) or Smart Shutdown (for overnight or unattended runs) is selected. At the end of your run, the system will then enter standby or

shutdown according to the settings defined in the instrument method configured earlier.

- Navigate to 'Queue'. Click 'Ready Check' to determine the readiness of the HPLC system.
- Click 'Start' to begin data acquisition if Ready Check returns no warning.
- Once the analysis is finished, your chromatography data will be automatically saved within your sequence. Refer to the HPLC PowerPoint workflow for guidelines on data processing.

### 5.3 Shut Down

- Remove your column from the column compartment and replace with a union fitting.
- Connect all eluent lines used during the run with bottles of fresh MilliQ water (e.g., if lines A1 and B2 were used, connect both to MQ water).
- Navigate to **Instruments > Home> Vanquish Pump**. Select the eluent lines used during the run from the dropdown menu.
- Set the % eluent for each eluent line accordingly.
- Toggle the flow on. Ensure the system backpressure remains within safe operating limits. For a total flow rate of 1 mL/min, the system backpressure should be around ~20 bar.
- Monitor the backpressure.
- Allow the system to flush for 1 hour. After 1 hour, stop the pump.
- Following a CAD detection session,
  - return the lineup to the default UV/RI detectors before concluding the session. This requires physically disconnecting the column outlet line from the CAD detector to the inlet of the UV detector.
  - Follow guidelines on slide 6 of the HPLC PowerPoint workflow to switch the instrument configuration from CAD to UV/RI.

### 5.4 Clean up

- Upon completion of the session, remove all sample vials from the autosampler and return all eluents and columns to your own laboratory for storage and disposal. Before leaving, ensure that a union fitting is installed in the column compartment.

### 5.5 Data transfer

- Personal data storage devices (e.g., USB drives) are not permitted for use with this instrument. Mediaflux Data Mover is the designated method for data transfer.

## 6 MAINTENANCE/TROUBLESHOOTING

- Operators shall keep the working area clean and tidy. Operators are not required for instrument maintenance and troubleshooting.
- Contact the instrument supervisor for any equipment-related issues.

## 7 INCIDENT/EMERGENCY

### 7.1 Incident

In the event of a minor incident (spill and cuts), operators shall report to the instrument supervisor or lab manager immediately.

**Chemical spills:** users shall use a spill kit and follow the clean-up procedure.

Any contact with chemicals, wash the area immediately and use emergency showers or eyewash for more serious incidents.

**Minor cuts:** use first aid kits properly if needed.

First aid assistance shall be provided by the area's first aid officer.

### 7.2 Emergency

In the event of a major incident/emergency (fire, explosion, medical emergency), operators shall:

- Notify the relevant emergency response department e.g. Fire, Ambulance, Police (dial "000").
- Notify the School of Chemistry OHS Coordinator (03 90355494)

## 8 REFERENCES

### 8.1 Legislation

Occupational Health and Safety Act 2004

Occupational Health and Safety Regulations 2007

### 8.2 Codes and Standards

AS/NZS 1337.1-2: Personal eye-protection

AS/NZS 1337.4-6: Eye and face protection

AS/NZS 2161 (Series): Occupational protective gloves

### 8.3 University Procedures/Guidance

The equipment is not considered as Regulated Plant.

See OHS' Regulated Plant Requirements

(<https://safety.unimelb.edu>).

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