***STANDARD OPERATING PROCEDURE – E004***

**High-pressure liquid chromatography (HPLC)**

1. **Objectives**

The objective of this document is to establish standard operating procedures for high pressure liquid chromatography, ensuring the safety of laboratory personnel by mitigating potential risks associated with hazardous materials, and injuries. Additionally, this SOP aims to enhance the efficiency of experimental workflows.

1. **Personal Protective Equipment**

To ensure safety of high-pressure liquid chromatography, appropriate personal protective equipment (PPE) must be worn. This includes:

* Long pants and closed-toe shoes with non-slip soles to protect against spills and slips.
* A long-sleeved, buttoned lab coat or gown to minimize skin exposure.
* Safety glasses or goggles to protect against splashes or flying debris.
* Disposable nitrile gloves to prevent direct contact with hazardous materials.
* If the user has long hair, it should be tied back.

1. **Potential Hazards**

High-pressure liquid chromatography poses various hazards that must be managed to maintain a safe working environment. These include:

* **Chemical Exposure:** Health risks from solvents and reagents (e.g., methanol, acetonitrile) that may impact individuals through skin contact, inhalation, or ingestion.
* **High-Pressure Hazards:** Risk of leaks, bursts, or equipment failure due to high-pressure liquid flow in the HPLC system.
* **Electrical Hazards:** Risk of electrocution, short circuits, or fires due to improper maintenance or faulty equipment.
* **Cuts and Lacerations:** Injuries caused by broken glassware during sample preparation or handling.
* **Puncture Hazards:** Risk of injury from the auto-injection system if hands or body parts are not kept clear.
* **Biological Hazards:** Contaminated samples (e.g., pathogenic microbials, clinical samples) can pose biohazard risks if not handled properly.
* **Fire and Explosion Hazards:** Flammable solvents (e.g., methanol, acetonitrile) can ignite if exposed to heat, sparks, or open flames.
* **Slip Hazards:** Spills of solvents or water can lead to slips and falls, creating safety concerns in the laboratory.
* **Environmental Hazards:** Improper disposal of solvents or waste can lead to environmental contamination.

**NOTE: All HPLC solvents and samples for analysis should be treated with care and the potential hazards associated with understanding.**

1. **Training**

Ensure all personnel have received proper training on their hazards and safe handling techniques.

* MC03 Chemical Safety II / Hazardous Waste Management
* MC07 Chemical Safety I / Chemical Safety for Laboratory Users

1. **Procedures**
2. Preparation

* The location of the closest spill kits, fire extinguishers, fire blankets, eyewashes, and safety showers should be known prior to work.
* Proper PPE should be worn to avoid contact with solvents used in HPLC.
* The safety data sheet (SDS) of all chemicals used should be read and understood prior to experimentation.
* Review the HPLC method, including sample types, mobile phase, and detection methods. If there are any uncertainties regarding the method used, contact the equipment custodian.
* Ensure all necessary materials and equipment are available and in good working condition.
* Prepare samples according to the verified protocols.
* Label all samples and reagents clearly.

1. Setup

* Ensure the HPLC system is clean and properly calibrated.
* Ensure all connections are secure and check for leaks.
* Fill the solvent reservoir with the appropriate mobile phase.
* When changing the mobile phase, the lines and columns should be washed with HPLC-grade water. Carefully check on the label for the maximum percentage of solvent that is acceptable by the equipment. Pump lines should be purged of all air bubbles and check for leaks before beginning any runs.
* Ensure that the pressure is behaving normally and is well below the maximum pressure for the HPLC system.
* Change the HPLC column according to the experiment.
* Turn on the HPLC system and allow it to equilibrate as per the method requirements.
* Ensure the waste solvent bottle / container attached to the equipment is not overflowing before the run.
* Ensure that all solvent bottles, reservoirs, and waste containers have secondary containment trays.

1. Operation

* The equipment should be monitored during the run in real-time for any irregularities (e.g. pressure fluctuations or leaks).
* Ensure the detector operates correctly and is set to the appropriate wavelength for detection.
* In case of a leak, spill, or equipment malfunction, stop the run immediately and follow spill response procedures.
* Ensure the waste containers are only filled with 70-80% of their capacity. Change the waste containers after the run.
* Some HPLCs have heaters and might generate excessive heat while running. Do not touch the HPLC during the run. Highly flammable material should be put away from HPLC.

1. Repairs and Certification

* The HPLC should be serviced regularly and inspected by qualified and / or certified individuals.
* Should mechanical or electrical faults be found, the machine should not continue to be used.

**6) Incident Reporting**

* Report any injuries to the Principal Investigator (PI) and / or departmental safety officer (DSO) immediately.
* For serious incidents, contact the Security Unit immediately by calling the 24-hour hotline on 2358 8999.

**7) References**

* Zaman, K. (2016). *SOP\_SMB019: High pressure liquid chromatography (HPLC).* Risk Assessment. The University of Sydney.
* Coleman, N. & Ahmad, Z. & Shepherd, N. (2014). *SOP SMB019.3 (ZA NC NS 0714): High pressure liquid chromatography (HPLC).* The University of Sydney.