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|   | <b>INSTRUMENT:</b> HPLC   | <b>PAGE NO.:</b> 01-02                   |
|   | <b>MAKE:</b> Cyber lab TM<br><b>MODEL:</b> LC-UV100<br><b>PROCURED ON:</b> 25-02-2008 | <b>EFFECTIVE DATE:</b><br>01/01/2022     |
|   | <b>SUBJECT:</b> <b>SOP FOR HPLC</b>   | <b>REVIEW PERIOD:</b><br>31/12/2022      |

**Objective:**

The following document describes the standard operating procedure for HPLC.

**Scope:**

HPLC system used for analysis and determination of sample components.

**Procedure:**

1. Turn on the UV detector for the HPLC you plan to use at least 10 minutes before running samples. The power switch is located on the front of the detector.
2. If the detector is already on, but the UV lamp is off, turn the lamp on by pressing “Shift” + “Lamp” two times.
3. If the “System is not calibrated” message is displayed, then the system must be recalibrated as follows:
4. Ensure that the flow cell contains UV/Vis neutral solvent. If you are unsure about this, consult with a qualified member of the Garner lab before proceeding to the next step. ▪ If the flow cell does not contain UV/Vis neutral solvent, flush the flow cell. Consult the 2489 Manual for the proper protocol.
5. Manually recalibrate the system by pressing “Shift” + “3” on the instrument panel. The detector should resume normal operation after this. If the system gives you an error message, power the unit off, unplug it for 5 minutes, then plug it back in and turn it on again. A partially open front cover on the UV detector will cause an error upon startup. Repeat the above instructions. If the detector does not calibrate, contact a Garner group member immediately.
6. Turn on pump power switch (s) located left side of the pump(s) for the same HPLC. o Special Instructions for the Waters 515 Pumps: Each pump needs to be configured to be run remotely by

the computer. To do this, cycle through the pumps' main menu with the arrow keys until you find the "Mode Local" setting. Press 'Edit/Enter' to change it to "Mode Rem." Then press the 'Run/Stop' button. Repeat this procedure for both 515 pump units. O

7. Turn on computer and login with given password.
8. Open "Empower 2" (icon on computer desktop) to run the HPLC system from the computer.
9. Login with ID and password.
10. Click "Run samples" icon on a new pop-up window.
11. Select project (Select an existing project or create a new project)
12. Select HPLC system. HPLC\_right for the 1525 Binary EF system (analytical or preparative) or HPLC\_left for the modular system (analytical only)
13. Click "OK." The system set-up ("Connecting to Empower Node 'Proline.')" will take a few minutes. If there is a communication problem between the computer and UV detector, reboot the computer. • If not using the Advion Mass Spectrometer, ensure that it is not connected to the HPLC

|                             | <b>PREPARED BY</b>                    | <b>CHECKED BY</b>                    | <b>APPROVED BY</b>                  |
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