

## Title: BioLogic LP Chromatography System Operating SOP

### Approvals:

Preparer:     Kari Britt     Date:     02Aug10      
Reviewer:     Sonia Wallman     Date:     02Aug10    

1. **Purpose:** Operation of the BioLogic LP Chromatography System.
2. **Scope:** Applies to the BioLogic LP Chromatography System for purifying proteins.
3. **Responsibilities:**
  - 3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
  - 3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
  - 4.1. BioLogic LP Chromatography System Instruction Manual
5. **Definitions:**
  - 5.1. CV: Column Volume;  $CV = \pi(L \text{ in cm})[(\text{radius of column in cm})^2]$
  - 5.2. L = Length of column (meaning the height of the bead bed)
  - 5.3. HETP: Height Equivalent to Theoretical Plate;  $HETP = L/N$
  - 5.4.  $N = 5.54 (t_R/w_{1/2})^2$
  - 5.5.  $t_R$ : retention time
  - 5.6.  $w_{1/2}$ : peak width at half height
  - 5.7. h: Reduced Plate Height;  $h = HETP/D_p$
  - 5.8.  $D_p$ : bead diameter
6. **Precautions:** N/A
7. **Materials:**
  - 7.1. deionized Water
  - 7.2. Equilibration Buffer A (Refer to the process SOP)
  - 7.3. Equilibration Buffer B (Refer to the process SOP)
  - 7.4. Cleaning Solution (Refer to the process SOP)
  - 7.5. biopure water
  - 7.6. container for waste fluid
  - 7.7. collection tubes for fraction collector or collection containers
  - 7.8. column (Amicon Vantage-L Biochromatography column and accessories)
  - 7.9. resin (Refer to the process SOP.)
  - 7.10. lab towels
8. **Procedure:**
  - 8.1. Turn on BioLogic LP system (switch is in the front, on the lower left side of the system).
  - 8.2. Turn on computer.
  - 8.3. Click on the LP DataView icon.
  - 8.4. Verify that the computer is communicating with the system as indicated by a green "Receive" circle on the upper right side of the computer screen.
  - 8.5. **Pump Calibration**

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- 8.5.1. Based on the desired flow rate, select the appropriate tubing for the pump as follows:

Flow rates of 0.04-0.8 mL/min require 0.8mm tubing.

Flow rates of 0.2-4.0 mL/min require 1.6mm tubing.

Flow rates of 0.8-15.0 mL/min require 3.2mm tubing.

- 8.5.2. Verify that the correct tubing is in the pump.
- 8.5.2.1. Remove the platen by lifting the grey handle (Figure 2).
  - 8.5.2.2. If necessary, insert the correct tubing.
  - 8.5.2.3. Replace platen and lock into place.
  - 8.5.2.4. If tubing was replaced readjust the platen and recalibrate the pump.
    - 8.5.2.4.1. Loosen the platen adjust screw located on the top of the pump (Figure 2) by turning counterclockwise until there is slight resistance.
    - 8.5.2.4.2. Tighten the platen screw clockwise the appropriate number of COMPLETE turns.
      - 0.8mm tubing requires 5 turns
      - 1.6mm tubing requires 4 turns
      - 3.2mm tubing requires 3 turns
  - 8.5.2.5. Recalibrate the pump.
    - 8.5.2.5.1. Press the MANUAL mode key.
    - 8.5.2.5.2. Press the PUMP instrument key.
    - 8.5.2.5.3. Select FLOW, then select CALIBRATE.
    - 8.5.2.5.4. Select the appropriate tubing size.
    - 8.5.2.5.5. Select NOMINAL.

### **8.6. Purge System with Buffer A and Zero the UV Monitor**

- 8.6.1. Place each buffer line into a container filled with Buffer A (Equilibration Buffer).
- 8.6.2. Attach the column inlet tube directly to the column outlet tube.
- 8.6.3. Press the MANUAL mode key.
- 8.6.4. Select BUFFER.
- 8.6.5. Select MIX.
- 8.6.6. Type in 50% B.
- 8.6.7. Select OK.
- 8.6.8. Select PURGE.
- 8.6.9. Allow system to purge until conductivity reading on the display panel of the Biologic LP system controller stabilizes (less than 5 minutes).
- 8.6.10. Select BUFFER.
- 8.6.11. Using the arrow key, select C.
- 8.6.12. Select OK.
- 8.6.13. Allow system to purge until conductivity reading on the display panel of the controller stabilizes (less than 5 minutes).
  - 8.6.13.1. While the system is running, zero the UV Monitor.
    - 8.6.13.1.1. Press the UV instrument key.
    - 8.6.13.1.2. Select ZERO.

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8.6.13.1.3. Verify that the absorbance changes to zero on the display panel of the controller.

8.6.13.1.4. Press the PUMP instrument key.

8.6.14. After conductivity stabilizes, select STOP.

### **8.7. Pour the Column (if necessary)**

8.7.1. Secure the column in an upright position to a stand using clamps.

8.7.2. Close the bottom valve on the column (handle should be in the horizontal position).

8.7.3. Add approximately 10mL Buffer A to the column.

8.7.4. Obtain the appropriate resin (Refer to the process SOP.).

8.7.5. Swirl the resin to make a homogeneous mixture.

8.7.6. Measure the appropriate amount of resin (Refer to the process SOP.) with a graduated cylinder and transfer to the column.

8.7.7. Dislodge any beads that stick to the column with additional buffer.

8.7.8. Position the 3 way valve on the top adapter to close the top port (handle points to the top port).

8.7.9. Place the tubing from 3 way valve into the waste container.

8.7.10. Secure the adapter housing to the glass column.

8.7.11. Allow the resin to settle until there is a clear layer of buffer above the surface of the resin.

8.7.12. Depress the top adapter until it reached approximately 3cm above the resin, making sure air and then liquid comes out the top of the 3 way valve.

8.7.13. Lock the adapter into place.

### **8.8. Attach the Column**

8.8.1. Position the 3 way valve to close off the column (handle points to the column).

8.8.2. Disconnect the column inlet and outlet tubing from the tubing connector.

8.8.3. Attach the column inlet tubing from the injector valve to the top of the column 3 way valve.

8.8.4. Attach the column outlet tubing to the bottom of the column.

8.8.5. Place tubing from the 3 way valve side port in the waste container.

8.8.6. Open the valve at the bottom of the column (handle in vertical position).

8.8.7. Press MANUAL mode key.

8.8.8. Select PURGE.

8.8.9. Allow buffer to drip into the waste container from the side port until air bubbles are completely absent from the tubing.

8.8.10. Simultaneously select STOP and position the 3 way valve to close the side port.

### **8.9. Pack the Column**

8.9.1. Place all lines in the appropriate buffers/solutions as per the process SOP.

8.9.2. Press the PROGRAM mode key.

8.9.3. Select LIST METHODS.

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- 8.9.4. Using the arrow keys, select the correct method (Refer to the process SOP.). If the method is not listed, refer to the Biologic LP Chromatography System Instruction Manual to create a new program.
- 8.9.5. Select OPEN.
- 8.9.6. Using the arrow keys, verify that the method agrees with the process SOP.
  - 8.9.6.1. If the method has been changed, refer to the Biologic LP Chromatography System Instruction Manual to edit the program.
- 8.9.7. Select DONE.
- 8.9.8. Press the RUN mode key.
- 8.9.9. System will have a 10 second delay.
- 8.9.10. Verify that the computer is recording data by the appearance of an S symbol on the graph.
  - 8.9.10.1. If the S is not present, click the “record” button on the toolbar on the computer screen.
- 8.9.11. Once the method is finished, unlock the top adapter, lower the top adapter down to ~2mm above the beds and then re-lock the adapter.
  - 8.9.11.1. Measure the bed height.
  - 8.9.11.2. Determine the Column Volume for your column using the following formula:  $CV = \pi(L \text{ in cm})[(\text{radius of column in cm})^2]$   
Refer to Definitions (section 5) as needed to complete the calculation.
  - 8.9.11.3. There is no need to save the chromatogram, clear the screen using the “clear” button on the toolbar.
- 8.10. **Determine the HETP and h**
  - 8.10.1. Attach an appropriate size sample loop to the sample valve (usually a 125 $\mu$ L loop is appropriate).
  - 8.10.2. Turn MV-6 injector valve knob counterclockwise until there is resistance.
  - 8.10.3. Draw 1mL of elution buffer into a syringe.  
Note: If the elution buffer does not contain salt, then sterile filter a 1M sodium chloride solution to inject into the system.
  - 8.10.4. Insert syringe into top port. Push slowly to fill sample loop while simultaneously collecting overflow in a beaker.
  - 8.10.5. Leave syringe in port.
  - 8.10.6. Turn MV-6 injector valve knob clockwise until there is resistance.
  - 8.10.7. Press the PROGRAM mode key.
  - 8.10.8. Select LIST METHODS.
  - 8.10.9. Using the arrow keys, select the correct method (refer to the process SOP). If the method is not listed, refer to the Biologic LP Chromatography System Instruction Manual to create a new program.
  - 8.10.10. Select OPEN.
  - 8.10.11. Using the arrow keys, verify that the method agrees with the process SOP.
    - 8.10.11.1. If the method has been changed, refer to the Biologic LP Chromatography System Instruction manual to edit the program.
  - 8.10.12. Select DONE.

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- 8.10.13. Press the RUN mode key.
- 8.10.14. System will have a 10 second delay.
- 8.10.15. Verify that the computer is recording data by the appearance of an S symbol on the graph.
  - 8.10.15.1. If the S symbol is not present, click the “record” button on the toolbar on the computer screen.
- 8.10.16. Once a full peak has been generated, stop the program.
- 8.10.17. Turn MV-6 injector valve knob counterclockwise until there is resistance.
- 8.10.18. Save and print the file, making note of the directory where the chromatogram was saved.
- 8.10.19. Clear the screen.
- 8.10.20. From the chromatogram determine the HETP and h.
  - HETP = L/N
  - Note: Use L in mm for this calculation.
  - $N = 5.54 (t_R/w_{1/2})^2$
  - $h = \text{HETP}/D_p$Refer to Definitions (section 5) and Figure 5 as needed to complete the calculations. Refer to the process SOP for the  $D_p$  value.

### **8.11. Run the Column**

- 8.11.1. Place all lines in the appropriate buffers/solutions as per the process SOP.
- 8.11.2. Press the PROGRAM mode key.
- 8.11.3. Select LIST METHODS.
- 8.11.4. Using the arrow keys, select the correct method as per the process SOP. If the method is not listed, refer to the Biologic LP Chromatography System Instruction Manual to create a new program.
- 8.11.5. Select OPEN.
- 8.11.6. Using the arrow keys, verify that the method has not been changed.
  - 8.11.6.1. If the method has been changed, refer to the Biologic LP Chromatography System Instruction Manual to edit the program.
- 8.11.7. Select DONE.
- 8.11.8. Press the “Run” mode key.
- 8.11.9. System will have a 10 second delay.
- 8.11.10. Verify that the computer is recording data by the appearance of an S symbol on the graph.
  - 8.11.10.1. If the S symbol is not present, click the “record” button on the toolbar on the computer screen.

### **8.12. Clean the Column**

- 8.12.1. Place buffer lines into the appropriate cleaning solution (Refer to the process SOP.).
- 8.12.2. Run the appropriate cleaning method (Refer to the process SOP.).

### **8.13. Clean and Store the System**

- 8.13.1. If the system will be used again with the same column within a few days, it may be stored “as is” after a run.

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- 8.13.1.1. Turn off the system and turn off the computer.
- 8.13.2. If the system will not be used within a few days it must be flushed with water then 20% ethanol and purged with air.
  - 8.13.2.1. Disconnect the column.
  - 8.13.2.2. Attach the column inlet tube directly to the column outlet tube.
  - 8.13.2.3. Place each buffer line into a container filled with biopure water.
  - 8.13.2.4. Attach the column inlet tube directly to the column outlet tube.
  - 8.13.2.5. Press the MANUAL mode key.
  - 8.13.2.6. Select BUFFER, then select MIX.
  - 8.13.2.7. Type in 50% B, then select OK.
  - 8.13.2.8. Select PURGE.
  - 8.13.2.9. Allow system to purge until conductivity reading stabilizes (less than 5 minutes).
  - 8.13.2.10. Select BUFFER.
  - 8.13.2.11. Using the arrow key, select C.
  - 8.13.2.12. Select OK.
  - 8.13.2.13. Allow system to purge until conductivity reading stabilizes (less than 5 minutes).
  - 8.13.2.14. Select STOP.
  - 8.13.2.15. Place each buffer line into 20% Ethanol and repeat steps 8.13.2.6. through 8.13.2.16.
  - 8.13.2.16. Place each buffer line on a lab towel or kimwipes so that they are open to the air and repeat steps 8.13.2.6. through 8.13.2.16.
  - 8.13.2.17. Turn off the LP Biologic System.

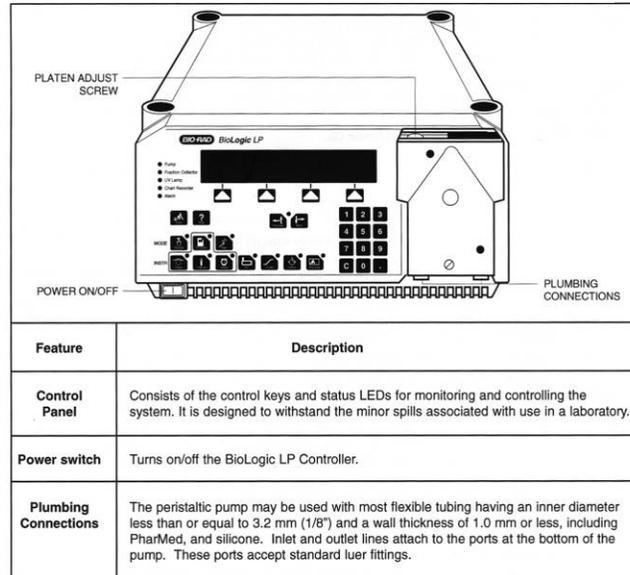
**9. Attachments:**

- 9.1. Figure 1: Controller Front Panel
- 9.2. Figure 2: Controller Pump
- 9.3. Figure 3: LP Biologic System Parts
- 9.4. Figure 4: Column components
- 9.5. Figure 5: Chromatogram example for calculating HETP

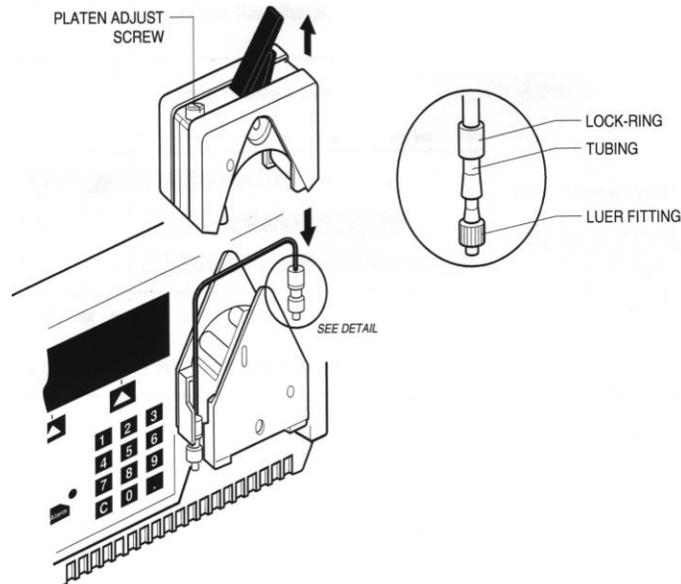
**10. History:**

Name	Date	Amendment
Deb Audino	070105	Initial release
Deb Audino	110405	Removed purging the system with water and Buffer B prior to use. Added the cleaning and storing section.
Deb Audino	17May06	Added the column components figure, added steps that were removed from the process SOPs.
Bob O'Brien	23Jan08	Added steps to clarify use of the 3 way valve.
Deb Audino	04Apr08	College name change
Kari Britt	03Aug10	Added to definitions and HETP sections. Added Figure 5. Made grammar and formatting edits as needed throughout the document. Removed references to programming SOP.

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**Figure 1: Controller Front Panel**



**Figure 2: Controller Pump**

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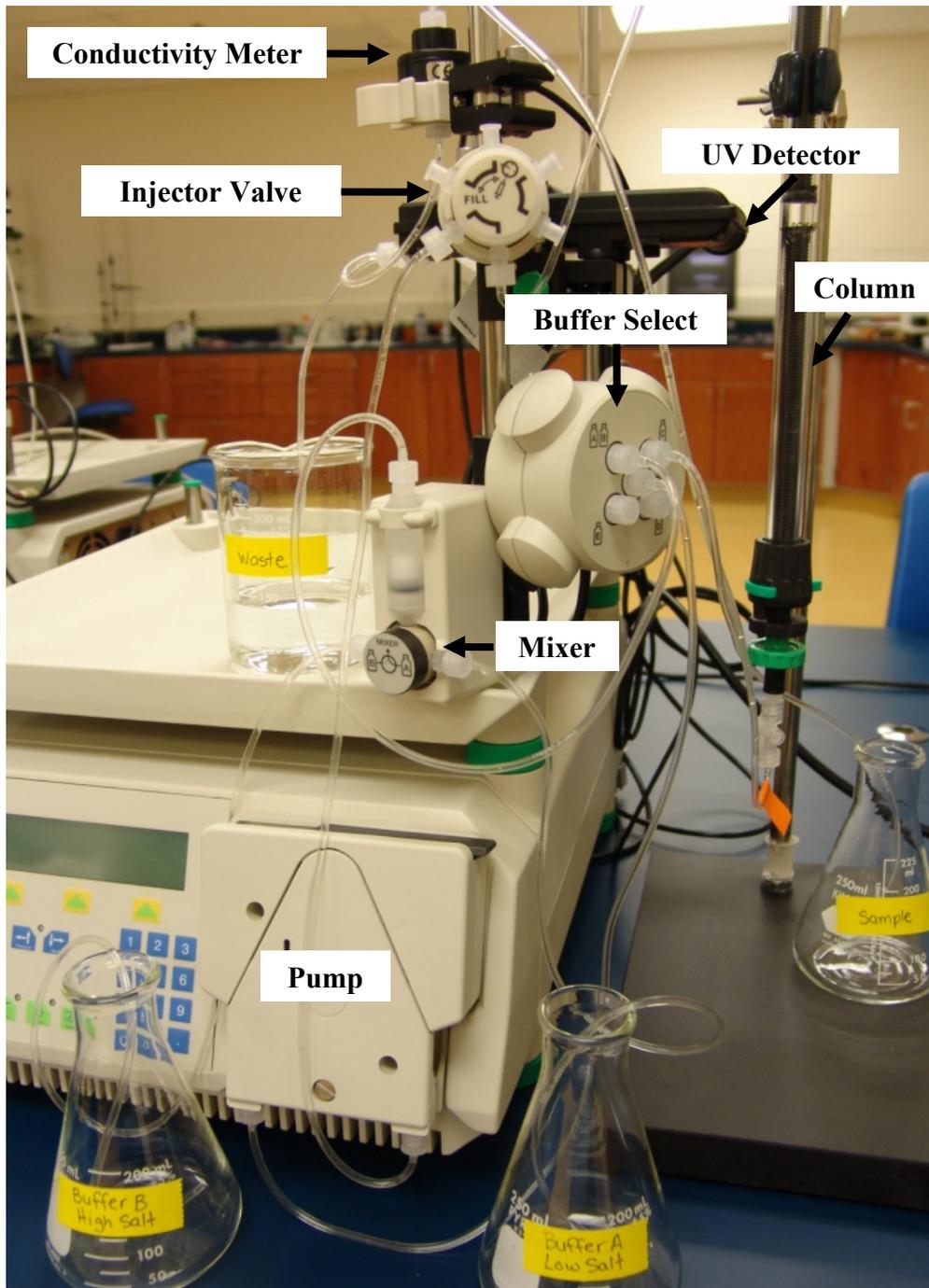


Figure 3: LP Biologic System Parts

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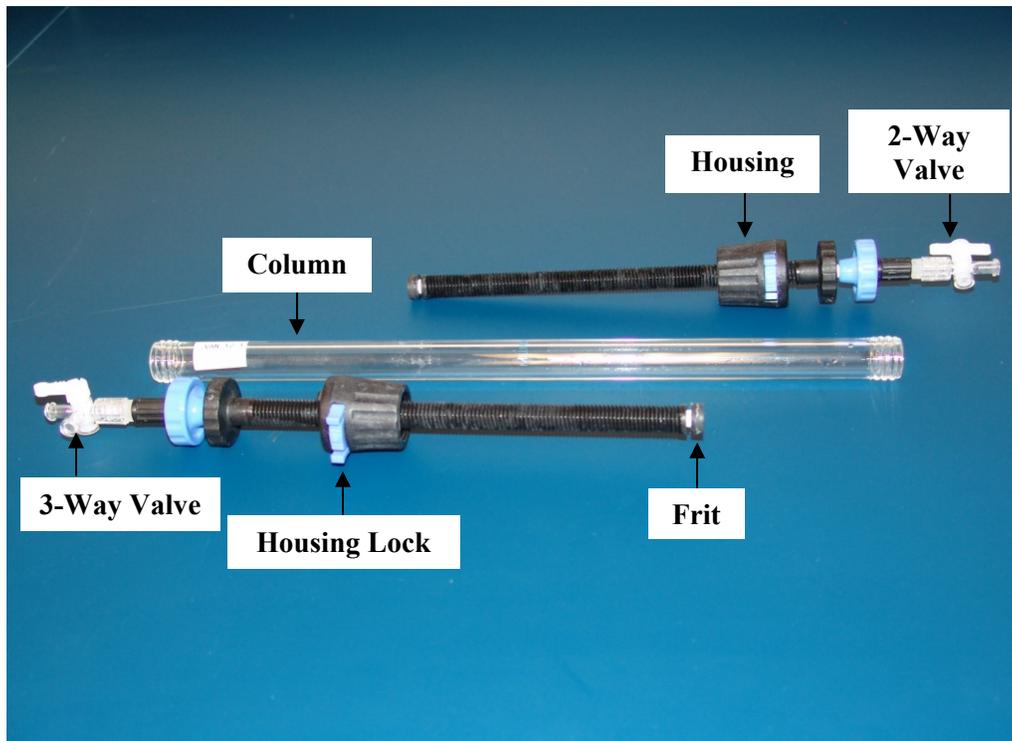


Figure 4: Column Components

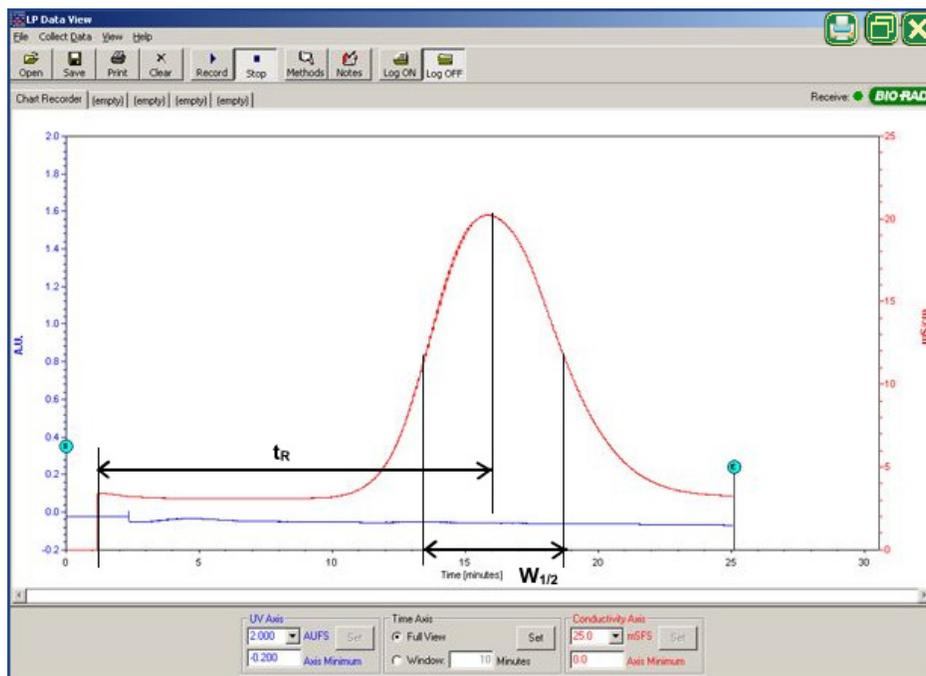


Figure 5: Chromatogram Example for Calculating HETP