

# Chromatography Techniques

## Course Outcome Summary

### Course Information

<b>Organization</b>	Madison Area Technical College
<b>Developers</b>	Lisa Seidman, Ph.D.
<b>Development Date</b>	1/20/1995
<b>Revised By</b>	Joseph M. Lowndes, Ph.D.
<b>Revised Date</b>	7/19/1995
<b>Course Number</b>	10-007-104
<b>Instructional Level</b>	Two-Year Technical Diploma
<b>Instructional Area</b>	Biotechnology Laboratory Technician
<b>Division</b>	Agriscience, Apprenticeship, Technical, Industrial
<b>Department</b>	Biotechnology Laboratory Technician
<b>Potential Hours of Instruction</b>	72
<b>Total Credits</b>	3

### Description

Introduces the basic concepts involved in separation of biomolecules. Students complete lab work using a variety of chromatographic methods including: paper, thin layer, gel permeation, gas and high performance liquid chromatography. Students also learn to interpret chromatographic results and practice documentation and reporting skills.

### Types of Instruction

Instruction Type	Contact Hours	Credits
A. Classroom Presentation	18	3
B. On Campus Laboratory and Clinicals	54	

### Textbooks

Bender. *Principles of Chemical Instrumentation*.

Ambrose and Ambrose. *Handbook of Biological Investigation*.

Biotechnology staff. *Resource Package*.

Biotechnology staff. *Laboratory Manual*.

### Learner Supplies

locker.

calculator.

lab coat.

lab notebook with carbon copy pages.

safety glasses and/or goggles.

### Prerequisites

Biotechnology Laboratory Skills for a Regulated Workplace (10-007-103)

Chemistry 1 (10-06-111)

Laboratory Math for Biotechnology (10-007-136) or satisfactory COMPASS Math Placement Test score  
OR consent of instructor

## Competencies

### Unit 1. BASIC CONSIDERATIONS AND TERMINOLOGY OF CHROMATOGRAPHY

#### A. Apply basic chromatographic theory

**Conditions - Competence will be demonstrated:**

- A.1. through the completion of written assignments
- A.2. through written examinations
- A.3. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

- A.1. learner examines the relationship between chromatography and biphasic separations.
- A.2. learner classifies chromatographic methods by type as explained in text and lecture.
- A.3. learner selects the appropriate chromatographic method for given circumstances.
- A.4. learner shows diagrammatically a stationary and mobile phase, column and fractions.

#### B. Interpret chromatographic results

**Conditions - Competence will be demonstrated:**

- B.1. through the completion of written assignments
- B.2. through written examinations
- B.3. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

- B.1. learner performs qualitative and quantitative analysis to determine the amount and type of an unknown sample when given a chromatogram that includes proper standards and information
- B.2. learner detects uncertainties in the analysis and points out ways to confirm the analysis

#### C. Judge the quality of a chromatographic separation

**Conditions - Competence will be demonstrated:**

- C.1. through the completion of written assignments
- C.2. through written examinations
- C.3. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

- C.1. learner calculates efficiency and selectivity, identify tailing and fronting
- C.2. learner uses these measures to analyze the ability of the method to separate a compound of interest when given a chromatogram

#### D. Explain the importance of values in bioseparation techniques

**Conditions - Competence will be demonstrated:**

- D.1. through the completion of written assignments
- D.2. through the completion of laboratory activities
- D.3. through written examinations
- D.4. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

- D.1. learner explains the goals of bioseparation techniques relative to analysis and production
- D.2. learner explains the importance of quality control and professional ethics in disciplines that use bioseparation techniques

#### E. Conducts lab preparation and record keeping

**Conditions - Competence will be demonstrated:**

- E.1. through the completion of written assignments
- E.2. through the completion of laboratory activities
- E.3. through written examinations
- E.4. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

- E.1. learner prepares run sheets in lab notebook before all labs
- E.2. learner maintains proper records in notebook during labs
- E.3. learner prepares formal lab reports on time

**Unit 2.**

**BIPHASIC SEPARATIONS**

**A. Apply the basic concepts of biphasic separation**

**Conditions - Competence will be demonstrated:**

- A.1. through the completion of written assignments
- A.2. through written examinations

**Criteria - Performance will be satisfactory when:**

- A.1. learner predicts the separation properties of a compound in a polar/nonpolar system based on the compound's properties
- A.2. learner explains the technique of organic extraction
- A.3. learner predicts separation properties quantitatively by applying a distribution coefficient equation

**B. Apply basic sampling principles**

**Conditions - Competence will be demonstrated:**

- B.1. through the completion of written assignments
- B.2. through the completion of laboratory activities
- B.3. through written examinations
- B.4. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

- B.1. learner documents sample collection
- B.2. learner assigns identification information to a sample
- B.3. learner samples a non-homogeneous mixture by collecting multiple sub-samples
- B.4. learner explains factors that govern sample collection

**Unit 3.**

**PLANAR CHROMATOGRAPHY**

**A. Perform paper and thin layer chromatography**

**Conditions - Competence will be demonstrated:**

- A.1. through the completion of laboratory activities
- A.2. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

- A.1. learner separates test compounds in lab and correctly identifies unknowns using thin layer chromatography
- A.2. learner separates test compounds in lab and correctly identifies unknowns using two dimensional paper chromatography
- A.3. learner keeps lab notebook with summary of procedure and all results

**B. Interpret results of paper and thin layer chromatography**

**Conditions - Competence will be demonstrated:**

- B.1. through the completion of written assignments
- B.2. through written examinations

**Criteria - Performance will be satisfactory when:**

B.1. learner explains the advantages of two dimensional chromatography

B.2. learner analyzes Rf values on chromatograms correctly

**Unit 4. OPEN COLUMN LIQUID CHROMATOGRAPHY/GEL PERMEATION CHROMATOGRAPHY**

**A. Run a silica gel column**

**Conditions - Competence will be demonstrated:**

A.1. through the completion of laboratory activities

A.2. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

A.1. learner pours silica gel column

A.2. learner packs silica gel column

A.3. learner runs silica gel column

A.4. learner successfully separates mixture of organic compounds

A.5. learner collects fractions

A.6. learner evaluates fractions

A.7. learner keeps lab notebook with summary of procedure and all results

**B. Run a gel permeation separation**

**Conditions - Competence will be demonstrated:**

B.1. through the completion of laboratory activities

B.2. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

B.1. learner pours a gel permeation chromatography column

B.2. learner packs a gel permeation chromatography column

B.3. learner runs a gel permeation chromatography column

B.4. learner successfully separates a test mixture into its components

B.5. learner collects fractions

B.6. learner evaluates the fractions using a spectrophotometer

**C. Interpret results of gel permeation separation**

**Conditions - Competence will be demonstrated:**

C.1. through the completion of written assignments

C.2. through the completion of laboratory activities

C.3. through written examinations

C.4. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

C.1. learner explains results in terms of molecular weight of sample components

C.2. learner identifies unknown correctly

C.3. learner evaluates chromatograms using measures in competencies 1.2 and 1.3

**D. Report results of gel permeation separation**

**Conditions - Competence will be demonstrated:**

D.1. through the completion of laboratory activities

D.2. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

D.1. learner documents results of gel permeation separation in a lab notebook with summary of procedure and all results

D.2. learner prepares formal report that summarizes interpretation of results in tabular and text form

**Unit 5. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY**

**A. Perform proper maintenance and operation techniques to HPLC system****Conditions - Competence will be demonstrated:**

- A.1. through the completion of laboratory activities
- A.2. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

- A.1. learner attaches proper column to system
- A.2. learner checks for leaks
- A.3. learner checks pressure
- A.4. learner primes pumps
- A.5. learner runs test sample
- A.6. learner stores column in correct storage solution
- A.7. learner identifies components of HPLC system and traces flow of liquid

**B. Run a sample on HPLC system****Conditions - Competence will be demonstrated:**

- B.1. through the completion of laboratory activities
- B.2. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

- B.1. learner obtains correct separation of sample components

**C. Interpret results of reverse phase column and sample interactions****Conditions - Competence will be demonstrated:**

- C.1. through the completion of written assignments
- C.2. through written examinations
- C.3. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

- C.1. learner explains results in terms of reverse phase column and sample interactions
- C.2. learner identifies unknown correctly
- C.3. learner evaluates chromatograms using measures in competencies 1.2 and 1.3

**D. Document and report results of reverse phase column and sample interactions****Conditions - Competence will be demonstrated:**

- D.1. through the completion of laboratory activities
- D.2. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

- D.1. learner documents results of reverse phase column and sample interactions in a lab notebook with summary of procedure and all results
- D.2. learner prepares formal report that includes summarizes interpretation of results in tabular and text form

**Unit 6. GAS CHROMATOGRAPHY****A. Perform gas chromatography using standard and capillary columns and two types of detectors****Conditions - Competence will be demonstrated:**

- A.1. through the completion of laboratory activities
- A.2. through the completion of written explanations of results and observation of laboratory activities

**Criteria - Performance will be satisfactory when:**

- A.1. learner separates test compounds in lab using both Gow Mac and Hewlett Packard gas chromatographs

A.2. learner correctly identifies unknowns

**B. Interpret results of gas chromatography**

**Conditions - Competence will be demonstrated:**

B.1. through the completion of written assignments

B.2. through the completion of laboratory activities

B.3. through written examinations

B.4. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

B.1. learner explains results in terms of sample and column polarity and interactions

B.2. learner correctly evaluates chromatograms using measures in competencies 1.2 and 1.3

**C. Document and report results of gas chromatography**

**Conditions - Competence will be demonstrated:**

C.1. through the completion of laboratory activities

C.2. through the completion of written explanations of results and observations of laboratory activities

**Criteria - Performance will be satisfactory when:**

C.1. learner documents results of of gas chromatography in a lab notebook with summary of procedure and all results

C.2. learner prepares formal report that includes summarizes interpretation of results in tabular and text form