

OHLONE COLLEGE
Ohlone Community College District
OFFICIAL COURSE OUTLINE

I. Description of Course:

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| 1. Department/Course: <u>CHMT - 104A</u> | 7. Degree/Applicability:
Credit, Degree Applicable, Not Transferable (D) |
| 2. Title: <u>HPLC</u> | 8. General Education: |
| 3. Cross Reference: HPLC BIOT - 104A | 9. Field Trips: <u>Not Required</u> |
| 4. Units: <u>0.5</u>
Lec Hrs: <u>0.25</u>
Lab Hrs: <u>0.75</u> | 10. Requisites: |
| 5. Repeatability: <u>No</u> | |
| 6. Grade Options: Grade Only (GR) | |

12. Catalog Description:

This course trains students in High Pressure Liquid Chromatography, a technique used to separate and analyze chemical mixtures. The course is designed for beginners and intermediate-level users in HPLC who want practical laboratory experience. The lectures, supplemented by problem sets, slides, and video presentations, provide the fundamentals needed to understand the techniques and instrumentation involved in this powerful analytical tool. Key topics include basic HPLC instrumentation, detectors, including UV/vis, photo diode array, column selection, qualitative and quantitative analysis and troubleshooting HPLC systems.

13. Class Schedule Description:

Learn to operate HPLC instrumentation; important for analyzing the composition of substances.

14. Counselor Information:

This course serves a variety of career tracks; important for students training to become technicians in chemical, biological, biotech, and environmental laboratories; also valuable for incumbent workers who wish to upgrade their skills.

II. Student Learning Outcomes

The student will:

1. Demonstrate proper protocols in turning on, warming up, conditioning and checking the system suitability of the HPLC machine.
2. Describe the purpose and function of the basic components of the HPLC, including the pump, injector, column, detector, degasser, and mobile phase reservoir.
3. Prepare the buffer for the mobile phase according to proper protocols.
4. Demonstrate proper protocols in sample preparation, loading samples, installation and maintenance of new HPLC columns, as well as complex separation of steroids, metabolites, organic compounds, additives in food and agricultural industry, pharmaceutical drugs, as typically encountered in industry.
5. Conduct measurement and verification of HPLC and column performance like peak tailing, peak asymmetry, number of theoretical plates, resolution, and efficiency.

6. Assess system suitability by viewing the results generated by automated software used in combination with the HPLC instrumentation.
7. Demonstrate proper troubleshooting protocols of the HPLC machine and components.

III. **Course Outline:**

HPLC Overview

Important HPLC fundamentals, principles and theory behind separation
Instrumentation overview -- pump, injector, column, detector, degasser, and the mobile phase reservoir
Basic HPLC hardware

Separation Fundamentals and operating parameters

Mobile phase, stationary phase, and additives
Mobile phase strength and composition (organic, aqueous, and buffers)
Detector wavelength
Injection volume
Auto-sampler temperature and temperature controls
Run time
Column temperature and effect of temperature on retention and selectivity
Flow rate
Retention times
Resolution between adjacent peaks, peak symmetry, peak tailing
Column efficiency, selectivity, and conditioning
Factors affecting separation
Column usage (column cleaning, maintenance, eluent types, and pH compatibility)
Gradient vs. isocratic elution

Separation modes and columns

Adsorbents, stationary phase, and column selections
Normal and reverse phase HPLC
pH and temperature effects in reversed phase and column dimensions

Quantitative and qualitative analysis

Preparation of samples and standard solution from stock solution
Making test injections and monitoring of chromatograms
Method development for a completely unknown mixture
Basic integration of the chromatograms
How to label the peaks using RTR (retention time ratio) and calculate tailing factor and system suitability
Integration of results and chromatograms
Data processing using compatible software
Different ways of integrating peaks when peaks are not well resolved
Common integration errors and how it affects the peak area and peak height measurements

Trouble shooting of HPLC methods

Hardware troubleshooting
Separation troubleshooting
Maintenance recommendations
Unusually high or low pressure
System leaks
Air bubbles
Column channeling

Decreased retention time
Baseline drift

IV. **Course Assignments:**

A. Reading Assignments

1. Textbook
2. Handouts

B. Projects, Activities, and other Assignments

1. Lab-based activities such as loading and analyzing of samples, checking system suitability, system parameters, system components, and troubleshooting.

C. Writing Assignments

1. The student will maintain a lab notebook.

V. **Methods of Evaluation/Assessment:**

A. Lab based exams and quizzes

B. Instructional laboratory monitoring and supervising and/or individual observations and conferences with students

C. Objective and practical evaluation of students' laboratory skills as demonstrated by their appropriate use of the HPLC.

D. Demonstration of students' lab skills in column selection, choosing appropriate HPLC parameters, conducting a successful separation(s) and troubleshooting.

VI. **Methods of Instruction:**

A. Lecture

B. Laboratory

C. Discussion

D. Demonstration

E. Audiovisual

F. Computer Assisted Instruction

G. Collaborative Learning

H. Other

1. Individual assistance

2. PowerPoint presentations enhanced by electronic notes made available on instructor's web page

3. Hands-on industry-based lab separations

VII. **Textbooks:**

Required

1. John Kenkel *Analytical Chemistry for Technicians* 3rd Edition, CRC Press, 2008

Optional

VIII. **Supplies:**

A. Goggles with indirect venting and side shielding

B. Lab coat

C. Lab gloves

D. Lab notebook

E. Scientific calculator

