I. Description of Course:

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

II. 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.

III. Class Schedule Description:

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

IV. 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
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**

    **Optional**

VIII. **Supplies:**
   A. Goggles with indirect venting and side shielding
   B. Lab coat
   C. Lab gloves
   D. Lab notebook
   E. Scientific calculator