FS 520

INSTRUMENTAL ANALYSIS: Theory and practical application of analytical and preparative instruments with emphasis on chromatography and mass spectrometry.

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Office Hours: Thursdays 9:30 - 10:30 am and by appointment.

The class will cover the theory and practical application of various methods used to analyze biological and chemical samples, including hands-on practice with equipment used in modern life science laboratories. The course will emphasize spectroscopic and chromatographic instruments and methods. Topics covered included: pre-experiment planning, preparative methods, analytical methods, statistical evaluation of data, and examination and presentation of results. The course also introduces students to basic of computer modeling and visualization of biological molecules.

The electronic version of handouts will be send as a attachment by email. In additions to two books: required - Francis Rouessac and Annick Rouessac, *Chemical Analysis, Modern Instrumentation, Methods and Techniques. Wiley 2000,, (ISBN 0-471-98137-0)***,** and recommended -James W. Robinson, Eileen M. Skelly Frame and George M. Frame II *Undergraduate Instrumental Analysis, 6th editions*, several computer programs will be used as training tools, including Phenomenex HPLC teaching programs, *(Introduction to GC, Introduction to HPLC, Separation Modes of HPLC, Equipment used in HPLC,* and *Method Development in HPLC)*. The Perkin-Elmer capillary electrophoresis (CE) teaching software will aid students' understanding separation modes used in CE. The J&W software will help to understand GC separation techniques.

Class also relies heavily on excellent website: http://www.chromacademy.com/. As student you can register and use ChromAcademy for free.

University of Idaho Classroom Learning Civility Clause:

In any environment in which people gather to learn, it is essential that all members feel as free and safe as possible in their participation. To this end, it is expected that everyone in this course will be treated with mutual respect and civility, with an understanding that all of us (students, instructors, professors, guests, and teaching assistants) will be respectful and civil to one another in discussion, in action, in teaching, and in learning. Should you feel our classroom interactions do not reflect an environment of civility and respect, you

are encouraged to meet with I during office hours to discuss your concern. Additional resources for expression of concern or requesting support include the Dean of Students office and staff (5-6757), the UI Counseling & Testing Center's confidential services (5-6716), or the UI Office of Human Rights, Access, & Inclusion (5-4285).

Objectives of Course:

1) Understand the principles of methods and analytical instruments used in analysis of biological samples including food products.
2) Be able to apply these principles including analytical instrument operation to analysis of sample in the laboratory situation.
3) Interpret data and to clearly communicate findings in the form of written reports.

Learning outcomes:

Upon successful completion of this course, students should be able to:

1. Define basic terms and concepts in scientific methodology of instrumental analysis of biological samples
2. Examine the chemical properties and characteristics of analyzed sample
3. Understand instrumental methods used for food and other biological samples
4. Describe and explain the principles of basic analytical techniques used for biological samples characterization
5. Upon completion of FS520 course, students should be able to use their background knowledge in organic chemistry, food chemistry and biochemistry to understand methodology that led or will lead in the future to key advancements in characterization of sample.

Assessment questions for the listed learning outcomes:

1) Compare and critically evaluate basic analytical instruments/methods used to analyze proteins, lipids, carbohydrates and minerals.
2) Describe principles of chromatographic, electrophoretic, spectroscopic or mass spectrometric analysis of basic ingredients of food sample.

Examinations:

Second Wednesday of February	Exam I	100 points
Third Wednesday of March (*practical*)	Exam II	100 points
First Wednesday of May (final)	Exam III	100 points

Final grade will reflect the average percentile of these three examinations. Exams will cover the assigned text reading and information presented during the lectures. Exam format will consist of five to 10 problem questions. The *practical* exam will include theoretical knowledge and practical operation of chosen instruments. For example student could choose from: GC/MS, HPLC/MS/MS, UPLC/MS/MS and MALDI/MS/MS, IC,

QPCR, various spectrometers, supercritical fluid extractor, and scintillation counter. Unexcused, missed exams will count as 0 points. Excused absences will be granted based on the individual cases. Refer to the University of Idaho General Catalog, Requirements and Academic Procedures, Section M. Acts of cheating or/and plagiarism in this class will result in an automatic 0 points for that exam or paper and could result in an automatic F as a final grade for the course. Refer to the Student Code of Conduct (2300) in the UI Faculty Staff Handbook for more information.

The University of Idaho course evaluation site is located at this URL: http://www.its.uidaho.edu/studentevals. Student evaluations are confidential. As soon as the student submits an evaluation, the data is entered into a table that cannot be linked to the student. I strongly encourage all students to take a time to take time to evaluate this class. The students' inputs help to make this better.

Topics to be covered:

1. Qualitative and quantitative analysis: sources of error in experimental results, examples of determinate and indeterminate errors, precision, accuracy, reliability of results: Gaussian's distribution, definition of mean and standard deviation.
2. Introduction to chromatography, principles of chromatography.
3. Efficiency of the chromatographic process, Van Deemter equation, theoretical plate concept. A PC database of gas-chromatograms viewing utility will be used to learn and compare different gas chromatography (GC) column applications in modern gas chromatography.
4. Gas chromatography, theory, and equipment.
5. Different detectors and columns used in GC and their application and modes of operation.

    - Demonstration of capillary and packed columns
    - Demonstration of flame ionization (FID) and electron capture (ECD) detectors
    - Demonstration of GC equipped with mass spectrometer detector (MSD)
    - Phenomenex animated PC software will be used to present the concept and applications of gas chromatography.

6. Liquid chromatography, detectors, normal phase versus reversed phase chromatography, ion exchange, gel permeation and supercritical fluid chromatography.

    - Preparative, analytical (narrow bore) and nano-bore columns applications
    - Demonstration of modern HPLC hardware and software
    - Demonstration of modern UPLC hardware and software

- Demonstration ion chromatography system
- Separation method development
- Phenomenex animated PC software will be used to present the concept and applications of liquid chromatography (LC).

7. Mass spectrometer detectors; quadrupole, time of flight and ion trap. Theory and demonstration of actual applications in GC/MS and HPLC/MS/MS analysis; interpretation of MS and MS/MS spectra.

    - Electron impact ionization (EI)
    - Analysis of positive and negative ions, concept of m/z
    - Chemical ionization (CI)
    - Electrospray ionization versus MALDI ionization if sample
    - Atmospheric pressure chemical ionization (APCI)
    - MS/MS, collision cell functions and tune page setting
    - Understanding MS/MS modes; daughter, parent, MS2, Q1F, neutral loss, neutral gain
    - Introduction to a hybrid Quadrupole - Time of flight (QTof) tandem mass spectrometer (QTof), Waters QTof Premiere proteomics analysis systems.

11. Spectrophotometry; (fluorometry, colorimetry, polarimetry, nephelometry, turbidimetry). The absorption laws of spectrophotometry. Demonstration of spectrophotometric equipment. Methods used in single-beam and double-beam spectrophotometry.

    - Demonstration of UV-Visible-NIR diode array spectrophotometer
    - Demonstration of Horiba FluoroMax-3 spectroflurometer

12. Radioisotopes in biological research.

    - Origin and properties of radioactivity
    - Detection and measurement of radioactivity
    - Liquid scintillation counting
    - Scintillation counting of γ ray
    - Concept of dpm and cpm
    - Safety rules for handling radioactive materials
    - Preparation sample for experiment
    - Performing experiment
    - Demonstration of different scintillation counters

13. Nuclear magnetic resonance (NMR). Properties of nuclei, molecular motion, effect of radiant energy on molecules, principles of quantum theory, quantization of nuclei in magnetic field, chemical shifts, spin-spin splitting in proton NMR will be discussed

    - Important parts of the NMR instrument and their functions
    - Typical NMR H^1, C^{13} spectra and their interpretation

- Application of, NMR in food analysis and biochemistry

14. Infrared absorption

- Requirements for infrared absorption
- Energy levels in vibrating and rotating molecules
- Equipment: IR and FT IR spectrometer (radiation source, monochromators, detectors, sample cells)
- Fourier transformation detector systems
- Analytical applications, IR spectra interpretation

15. Electrophoresis

- Theory of electrophoresis, methods of electrophoresis, PAGE, IF, CE
- Capillary electrophoresis methods: capillary zone electrophoresis (CZE), capillary isoelectric focusing (CIEF), capillary dynamic sieving (CDS), capillary gel electrophoresis (CGE), micellar electrokinetic capillary chromatography (MECC), capillary ion analysis (CIA)
- Demonstration of capillary electrophoresis equipment (BioRad - BioFocus 3000), experimental setup, method development, various applications.
- Perkin-Elmer capillary electrophoresis simulation PC software will be used to help students to understand separation modes used in this type of instrumental analysis.
- The usage of capillary electrophoresis to measure organic acid content in wine samples will be discussed

16. Centrifugation of biomolecules.

- Basic principle of centrifugation, centrifugal force, sedimentation coefficient, Svedberg units (S)
- Instrumentation for centrifugation: bench top, high speed, ultracentrifuges
- Application of centrifugation, analytical and preparative centrifugation
- Demonstration of Beckman high-speed centrifuge and ultracentrifuge
- Comparison of differential, zonal and isopycnic centrifugation methods
- Safety and rules of operation

17. Basis of computer aided 3D visualization and modeling of molecules

- Internet resources available for computer aided visualization of molecules
- Introduction IRIX 6.5 operation system on Silicon Graphic computer
- Introduction to Ecce and NWChem, products of Environmental Molecular Sciences Laboratory.
- Introduction to Insight II 3D molecular modeling software
- Introduction to Gaussian 98 and Gview