

DUKE 401L: ILLICIT SUBSTANCES ON DOLLAR BILLS



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Duke 401L Illicit Substances on Dollar Bills

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This text was compiled on 08/02/2025

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CHAPTER OVERVIEW

What's on Your Money? Designing Analytical Strategies for Trace Organic Residues

Learning Objectives

- Students will **describe the molecular-level models** of chromatographic separation and mass spectrometric identification that are relevant to their work.
- Students will **use models to explain** how chromatography and mass spectrometry separate and specifically identify trace organic material.
- Students will **use evidence from the literature** to design a protocol for sample preparation and a method to perform qualitative and quantitative analysis in the context of detecting and quantifying trace organic material.
- Students will **demonstrate competence with instrumentation and instrument software** to obtain and analyze chromatographic and mass spectral data.
- Students will apply chemical knowledge to **analyze and interpret chromatographic and mass spectral data** in order to identify compounds and quantify illicit substances on currency.
- Students will **communicate the broad relevance, specific goals, methods, results, and analysis** of their work in the form of a scientific manuscript.

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1.1: Introduction

What's in your wallet? If you're holding paper currency, you may also be holding trace amounts of illicit drugs. Studies suggest that up to 80% of U.S. banknotes carry measurable levels of cocaine—and some are contaminated with methamphetamine, oxycodone, or other organic residues. These substances are likely transferred through indirect contact, such as with contaminated counting machines or storage environments.

In this experiment, you'll investigate what's on your money. You will be responsible for designing your own sample preparation procedure and calibration strategy to detect and quantify organic contaminants on paper currency. You'll choose what compounds to target—such as cocaine, methamphetamine, or other trace substances—and apply analytical principles to decide which tools and techniques are best suited for isolating and identifying them. Your team will extract material from bills, analyze it using a sensitive detection method that supports compound identification through fragmentation data, and interpret your findings based on comparison with known standards and spectral databases.

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1.2: Week 1

Pre-lab Assignment:

The first two questions in this pre-lab assignment are designed to engage you in thinking about how organic compounds, such as cocaine, interact with paper currency at the molecular level. By analyzing the chemical structures of both the contaminant and the currency material, you will apply your understanding of intermolecular forces to explain why some organic substances adhere strongly and persistently to surfaces like paper. These foundational chemical principles will help you understand the behavior of trace organic compounds in real-world forensic and analytical contexts.

Questions:

1. **What molecular-level interactions contribute to the adsorption of cocaine onto paper currency?** In your response, include the chemical structures of cocaine and cellulose (the primary component of paper). Draw a representation of how cocaine may interact with the cellulose surface. Identify and explain the intermolecular forces (e.g., hydrogen bonding, van der Waals interactions, ionic interactions) that are likely responsible for the adsorption and retention of cocaine on the surface of currency.
2. **Make a prediction about what organic compounds you will find on your currency during this experiment,** using evidence to support your prediction. Use Web of Science or another literature database to research substances that contaminate currency, and might be found on your money.

The second group of questions in this pre-lab assignment will help you practice using scientific literature to justify key decisions in analytical method design. By exploring how researchers select instruments, prepare samples, and use internal standards, you will learn how to propose a method that balances accuracy, safety, cost, and practicality. These skills will support your ability to justify experimental choices in your scientific manuscript.

Questions:

Use a research database to access the articles cited below and add them to your Zotero Library. Use these papers as reference for selection of your analytical methods and sample preparation protocols. *You do not need to read these papers from start to finish. Read the sections of the papers that help you with this assignment.*

Papers:

- Parker, Patrick D., Brandon Beers, and Matthew J. Vergne. "What Is in Your Wallet? Quantitation of Drugs of Abuse on Paper Currency with a Rapid LC–MS/MS Method." *Journal of Chemical Education* 94, no. 10 (2017): 1522–26. <https://doi.org/10.1021/acs.jchemed.7b00196>.
- Negrusz, A, JL Perry, and CM Moore. "Detection of Cocaine on Various Denominations of United States Currency." *Journal of Forensic Sciences* 1972-2005 43, no. 3 (1998): 626–29. <https://doi.org/10.1520/JFS16193J>.

1. What instrument will you use?

1. What kind of instrument detector enables specific identification and quantitation of trace organic analytes?
 2. What kind of instrument component can separate compounds in a mixture so that they can be analyzed by the detector described in (a).
 3. Propose an instrument that you will use to detect and quantify trace organic compounds on your currency. Justify why this instrument is the right choice for specific identification and quantitation of very low concentrations of organic analytes.
2. **Suggest a procedure to prepare the sample;** the protocol (1) will extract organic material from currency, (2) is simple and low cost, (3) avoids the use of particularly hazardous solvents (like dichloromethane or chloroform), (4) takes less than 30 minutes to prepare a sample, and (5) does not destroy the currency.
3. In the two manuscripts above, **what internal standards were used** during calibration and quantitation?
4. **Will you use an internal standard?** Decide whether you think an internal standard is necessary. Justify your answer with evidence from the papers or from other reliable sources.

Dry-Lab Group Discussion:

In this discussion, you will collaborate with your research team to refine your understanding of how organic compounds interact with paper currency and you will refine your extraction protocol and analysis method. By drawing molecular interactions, evaluating extraction protocols, and discussing calibration strategies, you will apply chemical principles and literature evidence to make informed decisions about your experimental approach. This discussion should will be facilitated in small groups (2-4 students) by a member of the instructional team.

Instructions:

- Begin the session by grouping up and comparing the answers to your pre-lab questions in your group for ~5 minutes.
- Write your work on the board and try to come to consensus as a group.
- If you have trouble reaching consensus, consult another group for advice, or consult an instructor when they are available.

Goals for Dry-Lab discussion:

1. Team consensus about method choice: Which instrument is best for this application, considering availability of instruments in the undergraduate learning labs?
2. Team consensus about extraction protocol: How will you prepare the sample?
3. Team consensus on calibration strategy: Will you use an internal standard?

Group Discussion Prompts

1. **Predict the relative amounts of cocaine and methamphetamine you will find on your currency.** Using your pre-lab response as a starting point, draw on the board:
 1. A molecular-level picture of how cocaine is adsorbed onto cellulose-based currency. Include chemical structures and show the intermolecular interactions.
 2. Do a similar analysis for methamphetamine and cellulose. Do you expect methamphetamine to absorb onto cellulose as well as cocaine does? Justify your prediction using the molecular structures as the basis.
 3. Predict the relative amounts of methamphetamine and cocaine on paper currency.
2. **What instrument will you use, and what are the appropriate settings?** Consider the instruments to which our course has access, and use the literature methods as a starting place. How will you need to modify instrument settings to see them maximum number of possible contaminants?
3. **Discuss your proposed extraction protocol with your group,** consider the limitations stated below, and finally consult your instructors. Discuss what are the costs and benefits of the suggested approaches and come to a group consensus about what protocol your team will use.
 1. Limitations of the analytical method: For example, if using gas chromatography, the sample should be dissolved in a solvent with a low boiling point so that the solvent elutes quickly and does not interfere with detection of the analyte ions. If using liquid chromatography, common mobile phases are water, methanol, and acetonitrile.
 2. Limitations of analytical solution preparation: If the solvent is too volatile, it will be challenging to transfer quantitatively, and difficult to prevent evaporation (which would affect concentration).
 3. Limitations imposed by purchased standards: Since we cannot purchase these controlled substances without a license, the standards are purchased as dilute solutions. The standard solutions (cocaine, methamphetamine) are only available as dissolved in Methanol - this solvent is versatile and suitable for both GC and LC applications.
 4. Solubility: The solutes must be soluble in the solvent.
 5. Safety: Some solvents are not only flammable, but also particularly toxic. Do not use solvents that are particularly toxic, such as chlorinated solvents (chloroform and dichloromethane).
4. **Discuss your proposed calibration method with your group.** Specifically, discuss whether you think an internal standard should be used in calibration, and justify your response using evidence from analytical principles and the literature. Please consider the limitation that deuterated analytes are typically expensive.
5. **Write detailed protocols** for sample extraction and calibration strategy in your notebook to prepare for the wet-lab session.

Wet-Lab Agenda:

- 1. Lessons and Demonstrations:** Your TA will give a short lesson about the instrument you will be using for analysis. Draw diagrams of the instruments and equipment in your notebook.
 - Instrument: Draw a diagram of the instrument components in your lab notebook. Include and label the autosampler, sample, injector, the components of the chromatography system, and the components of the mass spectrometer.
 - Syringe-Driven Filter: Draw the syringe driven filter device in your notebook.
 - Autosampler vials: Draw a diagram of the autosampler vial.
- 2. Sample Preparation:** Confirm that all group members have the same plan for sample extraction. Record any observations and deviations from the planned protocol in your notebook and perform the sample extraction.
- 3. Instrument method:** Choose or write an appropriate method for analysis of your samples. Record the details of the instrument settings in your notebook.
- 4. Prepare sequence for sample injection.** Use the instructions in the instrument protocol to create a sequence file to inject the following:
 - A blank (the solvent matrix of your samples)
 - Sample(s)
 - A standard mixture
- 5. Group discussion about electron ionization mass spectrometry.** (while waiting for samples to run)
 - Search for cocaine mass spectrum using the NIST WebBook.
 - Identify mass ion (303), and two most abundant fragment ions (182, 282) of cocaine.
 - Explain how electron ionization created the mass ion and the fragment ions (all are $z=1$).
- 6. Prepare sequence for injection of calibration standards** to run overnight.
- 7. Export qualitative data figures and report.**
 - Create a table that lists the compounds found, their mass ions, retention times, and confidence in the assignments. An example table is below.

Example Table:

Compound	Ions identified	Found r.t. in standard (min)	Found r.t. in sample (min)	Confidence in compound identification
exampleamine	42, 84	3.42	3.43	94%
examplone	54, 154, 187	5.43	5.42	85%
methylexample	56, 74	Not present	4.38	80%

Reflection Prompts

- Briefly describe the extraction procedure your group developed. Explain the reasoning behind your choices, including how these choices can be justified using chemical principles and/or literature sources.
- Justify your group's decision about whether to use an internal standard in your analysis. If you chose to use one, explain how its chemical properties make it appropriate for this experiment. Support your reasoning using relevant chemical principles (e.g., structural similarity, retention time, ionization efficiency, or extraction behavior).

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1.3: Week 2

Pre-lab Assignment

This pre-lab is designed to prepare you for a productive dry-lab discussion and upcoming data analysis. By organizing your qualitative results and drafting your Methods section now, you'll be ready to share work-in-progress and get early feedback to improve your report.

Follow up from Week 1

1. **Complete and submit any unfinished business from the last meeting.**
2. **Begin preparing your manuscript document:**
 - a. **Complete a draft of your Materials and Methods section.**
 - b. **Complete the Qualitative Results section, including description and figures that show the results of qualitative analysis.**
 - Prepare a table of compounds identified in your sample.
 - Prepare a figure that provides data to support the identification of components of your sample.
 - The table and figures should complement each other.

The exercise below uses example data to help you practice choosing between linear and nonlinear regression models using quantitative evidence. This practice will prepare you to analyze your own calibration data in lab this week and make informed, data-driven decisions. Completing it in advance will help you fully engage in the upcoming lesson on quantitative analysis of GC-MS results.

Questions

Practice evaluating regression models for calibration data.

3. **Examine the example data sets below and determine whether a linear or nonlinear regression model is more appropriate in each case.** For each data set:
 - Fit both a linear and a nonlinear model (e.g., quadratic or exponential, as appropriate)
 - Compare the quality of the fits using appropriate error metrics (e.g., R^2 , residual plots, standard error, or percent error in calculated concentrations)
 - Justify your choice of model using quantitative evidence, not just visual analysis.

What patterns in the data would indicate that a nonlinear model may be more appropriate than a linear one? How would an inappropriate regression model affect your interpretation of an unknown?

Data Set A		Data Set B	
Conc. (ng/mL)	Response	Conc. (ng/mL)	Response
0.1	15	0.1	15
0.5	81	0.5	81
1.0	168	1.0	132
2.5	430	2.5	398
5.0	957	5.0	710
10.0	2397	10.0	1551
15.0	3986	15.0	2321
20.0	6384	20.0	2970

Hint

An appropriate analysis of fit should include a plot of residuals. You've practiced this before in your P-Chem coursework—reviewing your residual plots from the FTIR of HCl and the Apples Lab (Matlab scripts) may help refresh your memory and guide your approach here.

Dry Lab Group Discussion

In this session, you will work with your research team to improve your qualitative results section, and prepare for quantitative analysis of your calibration data. You will also share drafts of your Materials and Methods section to get early feedback from your peers. By analyzing example calibration data and refining your own data presentation, you will deepen your ability to justify decisions using chemical reasoning and statistical evidence. This discussion will take place with the whole team, and will be facilitated by a member of the instructional team. Please work with your team to follow the instructions below.

An instructor will come address questions as soon as they are available; if for some reason an instructor cannot help you during the session, we will address any questions reported out by your reporter before the end of the day.

Instructions:

- Assign the following roles, then follow the agenda below.
 - The recorder should record the conversation and questions on the board or in a shared electronic document.
 - The time keeper should set a timer with a loud noise to keep the group work on track.
 - The reporter will submit a photo of the white board or the shared document at the end of the period.

Agenda:

1. Discussion about how to interpret and show Qualitative Results - Use the Group Discussion Prompts to engage in discussion.
 - Time: **15 minutes**
 - Record: main points and questions that you have.
 - Prepare to discuss these points with your instructor when they become available.
2. Discussion about regression analysis - Use the Group Discussion Prompts to engage in discussion.
 1. Time: **15 minutes**
 2. Record: main points
 3. Prepare to discuss main points with your instructor.
3. Peer Review: Begin the session by peer-reviewing another person's document draft. Review the Materials and Methods Section and Qualitative Results section, adding constructive and critical feedback.
 - Time: **15 minutes**
 - Please be silent during this time so others can complete the assignment.
4. Discuss Materials and Methods - what questions came up as you reviewed other's work?
 - Time: **15 minutes**
 - Record: main points and questions on the board.

Group Discussion Prompts

Analysis of Qualitative Data:

1. Confirmation Through Multiple Lines of Evidence

- If two compounds have similar retention times, what additional evidence can confirm identity?
- How can you use both the retention time and fragmentation pattern to confidently assign identity to a peak?

2. Library Search Validation:

- What does the library match score tell you, and how reliable is it?
- How can comparing the MS of an unknown directly to the MS and retention time of a standard help confirm or challenge the library ID?
- What should you do if a peak in your sample does not match any in your standard?
- How would you communicate uncertainty in compound identification in your report?

Regression Analysis:

The form of a linear and a quadratic regression model is given below. Inspect these models:

$$\text{Straight Line: } y = mx + b \quad (1.3.1)$$

$$\text{Quadratic: } y = ax^2 + mx + b \quad (1.3.2)$$

The quadratic model includes more coefficients than a linear model, which gives it greater flexibility to fit the data—even if the underlying relationship is actually linear. This can lead to overfitting and the false impression that the quadratic model is "better." Even when a quadratic model is appropriate over a wide range of concentrations, a linear model might be more appropriate if your sample is at the lower end of the range, within a linear portion of the curve.

3. **Which statistical measures might mislead you into thinking a more complex model is better?**

4. **How can you know which to use?** What additional evidence can help you decide which model is truly more appropriate for your calibration data?

Goals for Dry Lab:

- Deepen your understanding of qualitative data:
 - Results section: Ensure your chromatograms and mass spectra are labeled and ready to use in your report, and that your description of the results is appropriate.
 - Discussion section: Prepare a strategy to write the Discussion of qualitative results.
- Improve your Materials and Methods section: Identify and address any gaps or unclear steps before you move forward with writing.
- Deepen your understanding of Calibration: Review how to evaluate regression model fit so you can apply those skills to your own calibration data later this week.

Wet Lab Agenda

1. **Access your data:** You initiated the calibration in Week 1. You will need to know your file names and in which directory you stored your data so you can locate the data and complete the analysis.
2. **Lessons and Demonstrations:** Your TA will give a short demonstration on how to perform quantitative analysis using the Agilent Mass Hunter Quantitative Analysis software
3. **Perform Quantitative Analysis:** You will work in groups to create a *batch file* and *method* for quantitative analysis
 - As a group, you will deliberate on the use of a straight line vs. quadratic regression model in order to most accurately represent your data. *In your lab notebook, make note of the results of any statistical or regression analysis used to come to a decision*
 - Produce a calibration curve using the regression model selected.
 - Perform quantitative analysis on your samples and standards. *Make sure to include error analysis!*
4. **Continue writing** the *Results* and *Discussion* sections of lab report (only after completing the reflection prompts below.)

Reflection Prompts

- **What regression model did you choose as the best fit for your calibration data?** Justify your choice using appropriate evidence.
- **In the sample data, how do the amounts of cocaine and methamphetamine compare to one another?** Rationalize this observation considering the molecular structures of cocaine, methamphetamine, and cellulose. You may also speculate about other factors that affect the presence of these substances on currency, as you see fit.

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1.4: Week 3

Pre-lab Assignment

This pre-lab is designed to prepare you to share work-in-progress and get early feedback to improve your report.

Follow up from Week 2

1. **Complete and submit any unfinished business from the last meeting.**
2. **Prepare your manuscript document:**
 - a. **Update the Materials and Methods section** based on last week's peer feedback.
 - b. **Update the Qualitative Results section** based on last week's peer feedback.
 - c. **Write your Discussion of Qualitative Results.**
 - d. **Write the Quantitative Results section, including description and figures that show the results of quantitative analysis.**
 - Prepare a table that communicates the results of calibration and quantitation.
 - Prepare one or more figures that shows calibration data.
 - The table and figures should complement each other.
3. Bring a **printed copy** of your manuscript to the Dry Lab meeting.

Dry Lab Group Discussion

In this session, you will peer review each other's reports and practice helping each other by giving impactful, actionable feedback.

Wet Lab Agenda

While you may not do any lab work in this session, please keep notes of your writing-related conversations in your lab notebook and complete the reflection prompts as usual.

1. **Revise Manuscript and Write Discussion, Conclusions, and Introduction Section.**
 2. **Meet with Writing Consultants.**
-

Reflection Prompts

- **How has writing about your lab experience supported your learning?**
 - **What have you done that has supported your learning in this module? What could you do better in the future?**
 - **What were the ways in which your instructional team supported your learning? What could they do better in the future?**
-

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