Gas Chromatography-Mass Spectrometry Quantitation Lab

1. Preparation for the Lab

Before the lab, all students must complete the online safety training (students will not be allowed into the lab without its successful completion) and quantitation homework.

Further, students will be required to complete a home-to-take test (the last section of this document). At the start of the lab, students are expected to have a mastery of the test material (being able to talk about it without reading. The lack of preparation will result in a delay in taking the lab to the next available time.

Students are expected to have their own laboratory notebook with page numbers (handwritten page numbers are acceptable). The safety glasses must be worn at all times in the lab (students should use their own; however, optionally, they may use those available in the lab).

2. Optional Topics

- Phthalates introduced to plastic (plastic toys) as plasticizers, regulated by EPA due to their toxicity.
- Fungicides (e.g., tebuconazole, propiconazole) used as preservatives in wooden products such as windows
- Essential oils (e.g., thymol, menthol, eucalyptol, carvone, carvacrol), in peppermint or savory.
- BTEX in gasoline, benzene, toluene, xylenes, ethylbenzene concentrations are sometimes used to aid in assessing the relative risk or extent of the contamination by gas stations
- Alkanes (C_6-C_{14}) in arson samples (or other matrices, e.g., fuels)
- Polycyclic aromatic hydrocarbons (PAHs), promutagenes found in charred meat products (or other matrices, e.g., fuels, air particles).

NOTE: Upon approval from the instructor, students may perform this assignment using their own analytes.

3. Introduction

Gas chromatography coupled with mass spectrometry (GC-MS) is an analytical method used for the Identification and quantification of numerous volatile compounds. The qualitative GC-MS analysis is conducted by matching the retention time of given unknowns and mass spectra of known chemicals, typically electron ionization (EI) mass spectra, with known standards or libraries. The quantitative analysis in the total ion current mode (TIC) can be performed as a traditional GC analysis based on the total peak area, however the correct approach to quantification is based on the peak area of a specific ion. In addition to the instrumentation, quantification is also dependent on the type of calibration method used. Various calibration methods, such as external standard calibration, internal standard calibration, and method of standard addition, may be performed. Today the most common in gas chromatography is the internal standard calibration. The essential component of the work in analytical labs in industry and regulation agencies is following the rules of Good Laboratory Practice (GLP). Thus, the underlying component of the lab is placed on sample labeling, organization of experimental work, and data recording.

The aim of this lab assignment is to evaluate GC-MS analyses using the internal standard calibration with selected compounds (corresponding to the 1st presentation) within two tasks.

In the 1st task, students will determine the instrumental limits of quantification and validate the method's repeatability based on the replicate determination of the targeted compound in an evaluation sample of a defined (known) composition.

This will be followed by the 2nd lab (upon successful completion of 1st lab), in which students will determine the concentrations in an unknown sample.

4. Overall Outline

Check the Blackboard for specific deadlines and grading rubric

	Sign up for the lab (topic and time) based on the timeline provided above to ensure the lab and instrument availability.
Take Home test	Complete home to take test, a lower score than 80% may lead to a delay of the lab to the next available time (one resubmission will be allowed).
Lab: Task I	The solutions preparation (stock solutions, calibration standards, and evaluation samples) and setup of GC-MS analysis sequence
	GC-MS analyses of samples in the instructor's laboratory. Students will be responsible for recapping their samples and sequence preparation; they also need to ensure that they have a record of the GC-MS method and sequence.
Intermediate Report	Data processing and submission of a Powerpoint and MS Excel file. A lower score than 80% may lead to a delay of the lab to the next available time (one resubmission will be allowed). The items to be addressed: a. Identification of compounds based on retention times and mass spectra. b. Generation of calibration data c. Validation of the method based on the quantified content of analytes in the evaluation samples and its comparison with the theoretically prepared concentration using the t-test (i.e., accuracy). Evaluation of repeatability of the GC analysis and also that of sample preparation (i.e., precision).
Lab: Task II	The unknown solutions preparation and setup of the GC-MS analysis sequence. If the calibrations from the 1st lab are correct and solutions are not evaporated, they can be reused for this lab (otherwise, they need to be remade).
	GC-MS analyses of samples in the instructor's laboratory. Students will be responsible for recapping their samples and sequence preparation; they also need to ensure that they have a record of the GC-MS method and sequence.
Final Report	Submission of the final report as PowerPoint and MS Excel files.
Lab Checkout	Return all cleaned tools and materials provided for the lab and waste disposal following lab safety protocol.

5. Instructions

Several "How to" videos are provided with detailed descriptions showing the sample, standard preparation, and data processing at https://arts-sciences.und.edu/academics/chemistry/kubatova-research-group/chrom_ms02.html.

5.1. Materials

Each student is responsible to have his/her own laboratory notebook with page numbers (handwritten page numbers are acceptable). The safety glasses must be worn at all times in the lab (students should use their own; however, optionally, they may use those available in the lab). Flashdrive is needed for data transfer and backup.

Students will be provided with gloves. If solutions are spilled on the gloves, students need to replace them.

For task 1 each student will receive two target compounds (corresponding to the 1st assignment) and an internal standard diluted solution. For task 2, an unknown sample will be provided. In the lab, solvents of GC or HPLC quality, including dichloromethane, *n*-hexane, and methanol will be available for sample dilution. At the beginning of each lab period, each student will also receive a check-out box, which must be returned to the instructor at the end of the lab period. This box will include volumetric flasks, two 10-mL vials, autosampler vials (2 mL), several syringes (10, 250 µL and 1 mL) for solution preparation, readymade internal standard solution, and Pasteur pipettes.

*Throughout the labs, all the flasks and vials must be **labeled with the student's initials** and the page number of the notebook on which the sample is described.* Omitting labeling of any samples will result *in a loss of points (5 pt per incident noted)*.

5.2. Preparation of Solutions

Video tutorials are provided for all solution preparation https://arts-sciences.und.edu/academics/chemistry/kubatova-research-group/chrom_ms02.html

Sample labeling: Each sample and solution must be labeled with the initials of the student preparing the sample, the page number in the notebook, and the number (or letter) referring to the location on the particular page. Omitting the labeling of the solutions and/or its recording the labels in the notebook will lead to the loss of points!!

Sample preparation for this project consists of the preparation of:

- stock solutions of the target analytes and internal standard (IS) (two analytes by each student)
- an initial calibration mixture (a) and its serial dilution (one set prepared jointly by two students)
- five evaluation samples (i.e., defined mixtures of two targeted analytes) prepared by each student
- triplicate unknown sample prepared by each student (Task 2)

5.2.1.Stock Solutions

For details on the experimental and organization of data in MS Excel, see videos (Section 10).

The students will prepare stock solutions of target analytes typically in a volume of 5-10 mL in volumetric flasks with final concentrations of 1-3 mg/mL, typically in dichloromethane (for more polar compounds, methanol may be used). Two stocks will be prepared by each student.

The internal standard (IS) should be a compound of similar chemical and physical properties as are target analytes and may NOT be naturally occurring in the samples. However, for the purpose of this lab, we will use a single IS, o-terphenyl diluted to ca. 5 mg/mL.

All of the stock solutions need to be prepared in volumetric flasks by weighing approximate amounts of the target analyte into the volumetric flask (e.g., ~5 mg for 5 mL flask) and adding the solvent up to the meniscus of the volumetric flask. The exact concentration should be determined after the sample preparation and pertinent calculations need to be shown in MS Excel. Each stock solution needs to be labeled and recorded in the lab notebook as described above.

5.2.2.Calibration Solutions

Note the solutions are prepared in dichloromethane, which has a low boiling point (37 °C) and thus evaporates very fast even at ambient temperature. Make sure that solutions between the sample preparation steps are closed/covered to ensure that the concentrations of stock solutions, standards, internal standard, and samples are not changing over time. In case of significant mistakes student may have to prepare solutions second time for 2nd task.

Student should first prepare from stock solutions 1 mL of mixture (A) consisting of 200 µg/mL (ppm) of each target analyte. For example, for stock solutions of target analytes in concentrations 3 and 5 mg/mL in stock solutions, student will need to use with 60 and 40 µL (round the volumes so they can be measured them) of each analyte, respectively. Then DCM is added, so the final volume is 1 mL. Using this preparation, the final concentrations of these analytes in mixture A will be 180 and 200 µg/mL.

The preparation of A mixture will be followed by the preparation of five additional calibration solutions using 5x fold serial dilutions (e.g., use 200 µL of mixture A + 800 µL of a solvent to prepare solution B, then 200 µL of mixture B + 800 µL of the same solvent to prepare solution C and so on). Thus 6 calibration standards of 800 µL. will be prepared *(note 200 µL must be removed and disposed from the lowest concentration standard)*.

For the internal standard (IS) calibration method, the IS is added to each standard solution (using the same volume/mass ratios). Thus same amount of IS (10 µL) should also be added to each calibration standards (A, B, C, D, E, F)(or other consistent volume if applicable).

5.2.3.Evaluation Samples

- The evaluation samples should be prepared as mixtures *by each student individually starting with a preparation of a new mixture, containing two targeted analytes (but not IS).* Note that this sample should have a different label in the lab notebook.
- From this mixture, three evaluation samples should be prepared by spiking the known volume of the mixture to the known volume of the solvent, thus resulting in 800 µL of the solution. The final concentration of each analyte should be ~20 ppm (the exact concentration must be calculated)**.**
- Then 10 µL of IS (or the same volume as added to the standard) should be added.

5.2.4.Unknown Samples (Task 2)

- Solution of unknown sample (~4.0 mL) will be given to each student. Student will take three 800 µL aliquots from the provided stock and add 10 µL of IS (or the same volume as added to the standard) to each to prepare three replicates for the analysis.

5.3. GC-MS Method

The experiments will be performed on an Agilent 6890N GC-MS system equipped with a split/splitless injector and a mass spectrometer (HP 5975C) with an electron ionization source, quadrupole analyzer and 7873 autosampler. The analyses will be performed on ca. 25 m long DB-5 (5%phenyldimethylpolysiloxane) capillary column with a 0.25 mm internal diameter and 0.25 µm film thickness.

Work in this section is to be performed with an instructor of the course or a graduate student (operator) in charge of the instrument. In the GC-MS logbook, record that this is a Chem 443 session, your name, date, the pressure in the He gas cylinder. The MS performance must be verified by the operator before the work in the lab checking for leaks and operation of the instrument using the testmix performance solution. Typically splitless testmix method can be used for analysis of most of the analytes, for BTEX solvent delay may need to be adjusted by the operator to a shorter time.

Record all operation conditions: including details about the instrument, temperatures of the injector and transfer line, the temperature program, the pressure on the used inlet, carrier gas and its flow rate, the split/splitless program, the volume of your sample injected (should be 1 µL) and MS conditions such as ionization source mass range for the total ion scan (TIC). *The conditions reported are essential to ensure that another chemist can repeat your work if needed.*

5.4. Setup of the GC-MS Sequence & Analysis

Work in this section is to be performed with the course instructor or a graduate student in charge of the instrument. To analyze samples using an autosampler, students must prepare a sequence (i.e., a table listing all standards and samples to be analyzed). Click "*Sequence/Sample log table"* to insert the samples, which will be analyzed. The sequence should be analyzed in the order specified below. The student needs to specify the sample name, file name (the filename should always start with the students' initials), name of the method, and vial position on the carrousel.

1) Blank (just DCM or solvent),
2) Performance Testmix (provided by the lab, run 3x)
3) Calibration standards from the lowest to the highest concentration
4) Evaluation samples for task 1 or unknown samples for task 2. To be able to evaluate the GC injection and sample preparation repeatability, analyze one of the samples three times and all other samples only a single time. Unknown samples should be analyzed only once (i.e., 3 analysis for each student)
5) Following the samples, reanalyze both calibration standard sets and testmix (if multiple groups are running samples, the testmix should be run only at the end).
6) Calibration standards (2nd time) from the lowest to the highest concentration

Save *"Sequence/save,"* name your sequence by YY-MMDD.

Before running the sequence, ensure that the vials with wash solvents on the autosampler are filled with methylene chloride. Recap samples as soon as possible to ensure that you can use the same samples for the analysis of unknowns (otherwise, you will have to prepare them again).

The operator of the instrument or the instructor will start the sequence.

5.5. Data Processing, Evaluation of Results and Report

Students need to copy the data files from the GC-MS computer need to be copied upon completion of the lab and can be processed in the computer lab on Windows-based computers in Chemstation or MassHunter software (check with the instructor for appropriate software selection. The related video tutorials are provided on this link: https://arts-sciences.und.edu/academics/chemistry/kubatova-research-group/chrom_ms02.html

5.5.1.TASK 1 Intermediate Report files

The Intermediate **Report PowerPoint and Excel files** must include a title page including the title of the lab "Gas chromatography-mass spectrometry quantification of……" name of students, and dates lab was performed. The file name should start with initials of the student. The files submitted following lab task 1 must include the following information:

1) *Identification of Analytes and Interpretation of Mass Spectra*

The first task of the project is to identify the compounds analyzed and reported as a single PowerPoint slide, including *the chromatogram, the mass spectra of target analytes and a brief statement* explaining the rationale for the Identification. Obtaining "clean" mass spectra may be helpful by subtracting the background.

2) Quantitative Data Analyses

Quantitative data analysis results should be reported in the Excel file, including calibration curves in TIC mode using the areas for specific ions and concentrations obtained for 2 calibrations (*do not average the data points*). The data processing in MS Excel should be done using both: the LINEST function and demonstrating calibration charts, see pertinent videos on LINEST, charts and LODs.

To generate the calibration curves for TIC GC-MS analyses, the student needs to obtain the peak area of each chromatographic peak using the extraction of characteristic ions. Higher accuracy and sensitivity are achieved when processing specific *m/z* ions instead of the total area. The justification statement of selected *m/z* ions used to quantify each analyte must be included in the report.

3) Validation of the Method Using the Evaluation Sample

All validation results should be shown in MS Excel spreadsheet supported by statements explaining the findings.

To validate the method, students need to calculate the analyte concentration in the evaluation samples based on the calibration curve and compare it to the true value (i.e., the concentration prepared) using the t-test at 95% confidence interval.

Furthermore, the precision of GC analyses vs. sample preparation should be evaluated based on comparing the average, standard deviations and relative standard deviations for concentrations of three replicate analyses of the same sample and the analyses of three different sample preparations, respectively.

Prior work on Task 2, student must submit the MS Excel and Power Point files to the instructor to receive approval to continue in the experimental work and if needed to determine remediation steps.

5.5.2. Final Report Files

The PowerPoint file will be considered as the main final report and must include the itemized below. The Excel file will provide supporting information in the form of calculations. Students must ensure clear slide titles and labeling to data to ensure clarity fo the report. The file name should start with the initials of the student.

1) *Title page including the title of the lab "Gas chromatography-mass spectrometry quantification of……" name of the students, and the dates the lab was performed*

2) *Introduction section, primarily based on the presentation assignment (2-3 slides)*

3) *Experimental section, including chemicals with their structure, description of analytical system with the operation conditions (2-3 slides)*

4) *Results and Discussion consisting of four sections (4-6 slides):*
    a. For the Identification of the target analytes, the slide should be prepared as for the task 1 report.
    b. Calibration results include charts of the linear curve for GC-MS and determined LOD and LOQs, including a statement comparing the obtained LOQs to those reported in the literature (1 slide)
    c. Validation of accuracy and precision of the GC analysis and sample preparation based on the analysis of the evaluation samples. These slides (1-2) should include repeatability based on the mean, standard deviation, relative standard deviation values, and conclusive statement.
    d. Characterization of the unknown sample including: i) identification of the major components supported by the mass spectra interpretation; and ii) quantification of the target analytes as mean,

SD and RSD along with a statement discussing possible implications regarding concentrations of other compounds identified.

5) *Acknowledgment*

6) *References in ACS format*

5.6. Lab Check Out

At the end of this lab assignment, each student will be responsible for returning the toolbox with cleaned syringes to the instructor. All disposable materials must be discarded, and glassware must be washed with water and ethanol. The samples and solvents should be disposed of in the properly labeled waste containers separating chlorinated and non-chlorinated waste. Each student needs to demonstrate proper use of the laboratory notebook to the instructor.

6. Grading & Report

The grading will be based on

(1) Prelab quiz (5 pt)

(2) Intermediate report submitted as Excel and PowerPoint files (10 pt)

(3) Final Report (60 points), consisting of the PowerPoint presentation and supported by MS Excel file sheet. The evaluation of the accuracy and precision of the work will be considered as equivalent of 20 points.

(4) Lab check out and demonstration of effective use of the lab notebook (5 pt)

7. Test Prior to the Lab

The answers must be provided prior to the lab; however, the student should have the knowledge the content without looking at notes. Lack of preparation will result in the cancelation of the lab and its delay to further date.

1. What is the purpose of this assignment?
2. What are the main tasks to be completed?
3. Explain the approach to internal standard calibration and its purpose.
4. What compound should be used for the internal standard quantification method?
5. What GC-MS temperatures and other instrumental operation values should you record in the report?
6. What is repeatability, and how is it evaluated?
7. What types of repeatability are evaluated in this work?
8. How do you prepare the evaluation sample in this lab (provide 3 step approach)?
9. What are the LOD and LOQ and how do you determine them?
10. How do you determine the accuracy of the method in this work?