JOURNAL OF
CHEMICAL EDUCATION

pubs.acs.org/jchemeduc

Laboratory Experiment

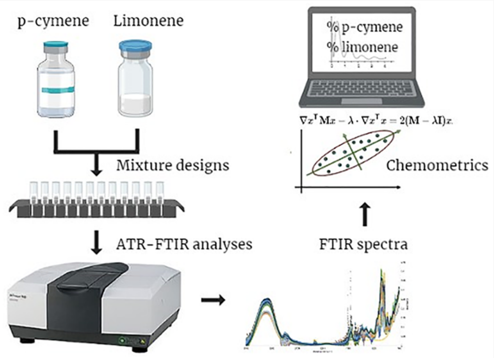
Chemometrics for Quantitative Determination of Terpenes Using Attenuated Total Reflectance−Fourier Transform Infrared Spectroscopy: A Pedagogical Laboratory Exercise for Undergraduate Instrumental Analysis Students

Gerard G. Dumancas,* Noemi Carreto, Oliver Generalao, Guoyi Ke, Ghalib Bello, Arnold Lubguban, and Roberto Malaluan

Cite This: *J. Chem. Educ.* 2023, 100, 3050−3060

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: Chemometric techniques such as partial least-squares (PLS) regression have been applied with huge success to a wide array of chemical problems including multicomponent analysis of analytes in complex mixtures. Despite this, there are few examples of laboratory pedagogical exercises that involve students in the acquisition of chemical data from infrared spectroscopic-based instrumentation, followed by quantitative chemometric analysis using PLS. In this article, we present a computational activity that introduces undergraduate students in an instrumental analysis laboratory setting to data acquisition using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy followed by data analysis using PLS. The first part of the activity involved students creating mixture designs of binary terpene components consisting of *p*-cymene and limonene. These mixtures were then analyzed using an ATR-FTIR spectrometer, where students became familiarized with the instrument and were shown how to characterize and differentiate the aforementioned terpenes using their generated FTIR spectra. The second part of the activity involved preprocessing the acquired FTIR spectral data from the first part followed by the simultaneous quantitative determination of the prepared terpenes using PLS. On the basis of student surveys, it can be concluded that this convenient and inexpensive activity was ultimately successful in introducing students to the use of chemometrics for quantitative analysis of terpenes using an ATR-FTIR. This easy-to-do two-week activity can be used as a standalone for instrumental analysis laboratory classes and can even be integrated in advanced courses in applied spectroscopy and chemometrics.

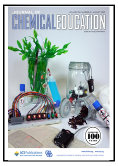
KEYWORDS: *upper-division undergraduate, analytical chemistry, computer-based learning, chemometrics, IR spectroscopy, spectroscopy*

■ INTRODUCTION

Even though the concept of Fourier transform infrared (FTIR) spectroscopy has been known for more than a century, it took more than 60 years for its application to be widely recognized as a potential analytical tool.[1] Today, due to rapid commercial development and extensive research, it is considered to be one of the most powerful chemical techniques, and has had a wide array of successes in agriculture, biomaterials, chemistry, and environmental science among others.[2−5] A wide variety of experiments have been introduced to students allowing them to learn more about FTIR spectroscopy, specifically on the use of an attenuated total reflectance (ATR) accessory for acquiring the infrared spectra of unknown solid and liquid organics, of inorganic solids synthesized by students, studying the adsorption of ions from solution onto oxide particle surfaces, and structural analysis of nanofilms.[6−8] Further, a variety of approaches have also been introduced to students to enhance their knowledge on spectral interpretation and understanding the FTIR such as by using a spreadsheet exercise, computing the infrared spectrum using the interferogram data, and by visual spectra comparison and statistical analysis and matching.[9−11]

Similar to FTIR, although there are existing laboratory exercises implementing the use of chemometrics, a highly interdisciplinary field, which employs applied mathematics, computer science, and multivariate statistics using methods employed in core-data analytic problems,[12] the majority of these are primarily targeted toward classification purposes (i.e., principal component analysis, factor analysis).[11,13−16] Further, although the chemical education community acknowledges the need for student training in various computational meth-

Received: April 21, 2023
Revised: June 27, 2023
Published: July 5, 2023



https://doi.org/10.1021/acs.jchemed.3c00358

ods,[17−19] there are limited pedagogical materials and no simplified or standardized way of incorporating these into the curriculum, especially in the use of instrumentation for data generation and consequent chemometric quantitative analysis of the same data. There are some studies, however, that are dedicated to the use of instrumentation and chemometrics for quantitative determination of analytes but are typically introduced in advanced analytical courses.[20,21] Further, many of these studies cannot also be directly implemented in the undergraduate curriculum because of the limitation of having to purchase expensive and not so user-friendly chemometric software packages.

In this article, we report a pedagogical approach for introducing chemometrics to the instrumental analysis curriculum by utilizing experimental data from FTIR analysis. Herein, we focus our analysis on monoterpenes, *p*-cymene and limonene (Figure S1), main components of essential oils that are known to have anticancer, analgesic, immunomodulatory, vasorelaxant, neuroprotective, and antinociceptive properties.[22−25] The purpose of focusing on a binary mixture is primarily due to advantages related to cost (i.e., *p*-cymene and limonene are readily available in many laboratories) and time (i.e., the project can be completed in 12 h).

In this two-week activity, students prepare binary mixtures of *p*-cymene and limonene and analyze each of the prepared mixtures using an ATR-FTIR in the first week. In the same week, students also get acquainted with the use of an ATR-FTIR as well as interpret the spectral peaks generated to distinguish the two terpenes. In the second week, students are introduced to chemometrics using partial least-squares (PLS) and then construct multivariate calibration models to simultaneously quantify the above-mentioned terpenes from the data generated in the first week. The PLS method is an exceptionally powerful chemometric approach that generates a calibration model of superior strength when compared to alternative techniques like multiple linear regression.[26,27] Moreover, it facilitates the examination of highly intricate mixtures, necessitating solely on knowledge of components of interest for constructing the calibration model. It accomplishes this by conducting regression utilizing latent variables or factors derived from the dependent and independent data, which align with their axes of highest variation and demonstrate maximal correlation.[27]

We have incorporated this activity in the instrumental analysis laboratory, although this could also be implemented in the quantitative analysis laboratory as well as advanced analytical chemistry classes. The code needed for the chemometric quantitative analysis was provided using an R-based Markdown document using a free available R package called the “pls”.[28] R is a freely available software package for statistical computing that runs on a wide variety of UNIX platforms, Windows, and Mac Operating Systems.[29]

■ METHODS

In this section, we present an overview of the methods used in this study. Additional background including student handouts and written statistical codes are readily available in the Supporting Information section. In this study, participants ($n = 20$) consisted of students with Environmental Science (15%), Forensic Chemistry (35%), Biochemistry (40%), and Chemistry (10%) majors.

Part 1: Preparation of Sample Mixtures and ATR-FTIR Analyses

Preparation of Terpene Mixtures. To obtain the data needed for the chemometric quantitative analysis of terpenes, the first week was dedicated to designing an experiment consisting of binary mixtures of *p*-cymene and limonene. In order to do so, a full factorial design was used to generate such terpene mixtures in four levels (i.e., low, average, above average, and high levels of terpenes) (($2^4 = 16$ mixtures) (Table S1)) using the "DoE.base" package in RStudio.[30,31] A full factorial design was used because it provides the experimenter sufficient information to evaluate the whole set of main effects as well as all interaction effects.[32] Further, it permits studying the effects of various factors on a response.[33] Due to the interest of time, an activity involving the implementation of the full factorial design using the aforementioned RStudio package was not covered in this lab. As such, a readily available template spanning the above-mentioned volume levels of terpenes was provided to the students (Table S1). For each mixture, specific volumes of each terpene (Table S1) were first added using a micropipet in a scintillation vial. Each mixture was then dissolved in 10 mL of ethanol, shaken by hand, covered tightly using aluminum foil capped scintillation vials, and immediately stored in a refrigerator to avoid evaporation. Since three trials were needed for each mixture for an ATR-FTIR analysis, pairs of students forming a group were each assigned to prepare 2−3 mixtures. This arrangement allowed us to finish all spectrophotometric analyses in 1 week given that all experiments were performed using the same instrument.

Qualitative Analysis of Terpenes by ATR-FTIR. An ATR-FTIR analysis was separately performed for each pure terpene to identify unique spectral regions for each of the analytes. Analysis was performed using a Shimadzu IRAffinity-1S with air as a blank. Each analysis was done in triplicates from 4700.52 to 339.47 cm^{-1} range ensuring that the ATR accessory was thoroughly cleaned, and background spectrum was taken before any sample spectral measurement. All analyses were performed at every 2 cm^{-1} resolution. The instrumental parameters/conditions included an absorbance measurement mode, Happ-Genzel apodization, measurement at 45 scans, and standard detector as an internal beam. The average of the spectral measurements was then used in the final data analysis. Peak picking was performed to identify the peaks in each prepared mixture. This was then correlated with the peaks of the pure terpenes to identify subtle signals due to each terpene component.

Part II. Chemometric Analysis of Terpene Mixtures

Quantitative Determination of Binary Terpene Mixtures Using FTIR and Chemometrics. The RStudio program was used to analyze the prepared mixtures from the previous part.[31] Since it is anticipated that students would have no prior experience in any kind of programming language, a brief introduction on how to run simple exercises using PLS and signal processing on RStudio was introduced to the class.[31] Specifically, the class utilized examples from running PLS using freely available gasoline data.[34] All PLS analysis was performed using the RStudio “pls” package.[28]

Data generated from the FTIR were preprocessed using the “prospectr” package in R using the first-derivative function.[35] First derivative was used in this study because it offers advantages of being able to resolve overlapping peaks, reduce baseline offset, compensate for instrumental drift, and enhance small spectral absorptions as well as provide predictions with increased accuracy.[35] Multivariate calibration, regression, and testing were performed using PLS, a powerful multivariate statistical technique that has been successfully applied in many areas.[36−40] To assess prediction accuracy of the regression model, the root-mean-square error (RMSE) was calculated as[41]

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{N} (y_i - y_i')^2}{N}}$$

where y and y' are the predicted and actual concentrations, respectively, and N is the number of samples. The R^2, goodness of fit between the predicted and measured values in the training set, was also calculated (Table S2). A visual overview of the two aforementioned steps is provided in Figure 1.

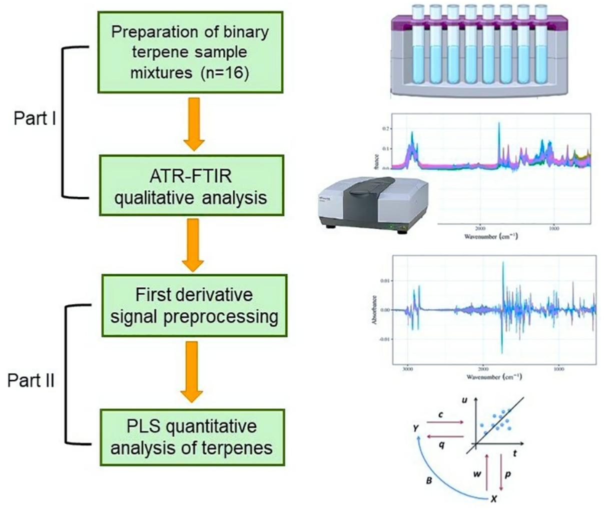


Figure 1. Overview of attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy and partial least-squares (PLS) quantitative analysis workflow for the simultaneous determination of *p*-cymene and limonene in binary mixtures of terpenes.

Chemical Hazards

Ethanol is a clear, colorless liquid that is absorbed by the gastrointestinal tract and distributed throughout the body. It may irritate or burn eyes and skin upon inhalation and contact with it. Further, its vapors may also cause asphyxiation or dizziness.[42]

p-Cymene inhalation causes impairment of coordination, andheadache. Contact with liquid causes mild irritation to the eyes and skin. Lastly, ingestion causes irritation of mouth and stomach.[43]

Limonene is a colorless liquid with a citrus-like odor. The liquid is known to irritate the eyes, and prolonged contact with skin causes irritation. Further, ingestion causes irritation of the gastrointestinal tract.[44]

Avoid inhalation and contact with these chemicals and ensure that all operations are to the extent possible performed under a certified chemical fume hood or another approved ventilated enclosure. Use safety goggles, masks, and a laboratory coat when using the aforementioned chemicals.

■ RESULTS

Vibrational Spectral Analysis of Pure p-Cymene and Limonene by ATR-FTIR

Each spectral measurement was thoroughly investigated by identifying peaks that differentiated one terpene from the other. The FTIR spectra of the *p*-cymene is characterized by unique peaks at 2957, 1514, 1101, 1055, 813, 719, and 540 cm^{-1}. The *p*-cymene spectrum displays the C═C stretching motion of the aromatic ring within the range 1500−1400 cm^{-1}, along with the symmetric bending vibration of the isopropyl methyl group at 1381 cm^{-1}. Additionally, the asymmetric vibration of the same group can be observed at 1362 cm^{-1}. On the contrary, the para-substituted phenyl moiety of the compound is evident within the spectral region of 1055−1019 cm^{-1}, whereas the robust double bond at approximately 813 cm^{-1} is attributed to the breathing mode of the phenyl nucleus. The morphology of the peak is likely influenced by the distinct substituents present on the para-substituted phenyl group (Figure 2).[45−47]

Limonene, on the other hand, has unique spectral regions found at 2913, 2832, 1643, 1435, 1375, 1150, 885, 799, and 430 cm^{-1} (Figure 2). This compound is characterized by C═C belonging to an alkene in the ~1643 cm^{-1} peak. Further, it is also characterized by CH (sp^2) and CH (sp^3) in the spectral range of 3012−3084 and 2966−2836 cm^{-1}, respectively. Other remarkable bands include ~1375 and 1453 cm^{-1} belonging to the CH$_2$ and CH$_3$ bends, respectively. Lastly, there was also a bending vibration of ═C−H of disubstituted and trisubstituted double bonds at 885 and 799 cm^{-1}.[48] The unique spectral differences among these pure terpenes clearly indicate that subtle differences would be sufficient to distinguish one terpene from the other in mixtures. The characteristic key absorbance bands among these two terpenes can be used for discriminating them (Figure 2). Such subtle differences may make it difficult to delineate the differences among the two terpenes in binary mixture designs in ethanol (Figure 3). As such, we utilized PLS regression to develop predictive models.

ATR-FTIR Data Preprocessing and Chemometric Analyses

Prior to the lab, data analysis was originally done by the instructor using the full wavenumber range (4700 to 339 cm^{-1}) to test the performance of the generated results using PLS. However, the results did not generate good results. As such, the instructor had also performed a prior analysis to test different wavenumber ranges, including 4700−339 cm^{-1}, 3225−428 cm^{-1}, 2261−428 cm^{-1}, 1875−525 cm^{-1}, 1585−525 cm^{-1}, 1489−718 cm^{-1}, 1489−525 cm^{-1}, and 1296−718 cm^{-1}, with the overall goal of optimizing the results while harboring the unique spectral peaks of each of the individual terpenes in the binary mixtures. In this activity, students tested various aforementioned wavenumber ranges to see how results changed. The results presented herein show the optimized results using the 1489−718 cm^{-1} range harboring many unique spectral peaks present in *p*-cymene and limonene, as mentioned earlier and was performed by the students. The developed calibration model in this specific spectral range (i.e., 1489−718 cm^{-1}) (Figure 4) generated three components for both *p*-cymene and limonene (Figure 5), which was sufficient to develop a robust PLS calibration model.

Further, these models yielded cross-validated root-mean-square error (RMSECV) values of 0.0944 for the given number of components mentioned previously for both the *p*-cymene and limonene terpene components. The developed model also yielded R^2 = 0.683 for both components, indicating moderate

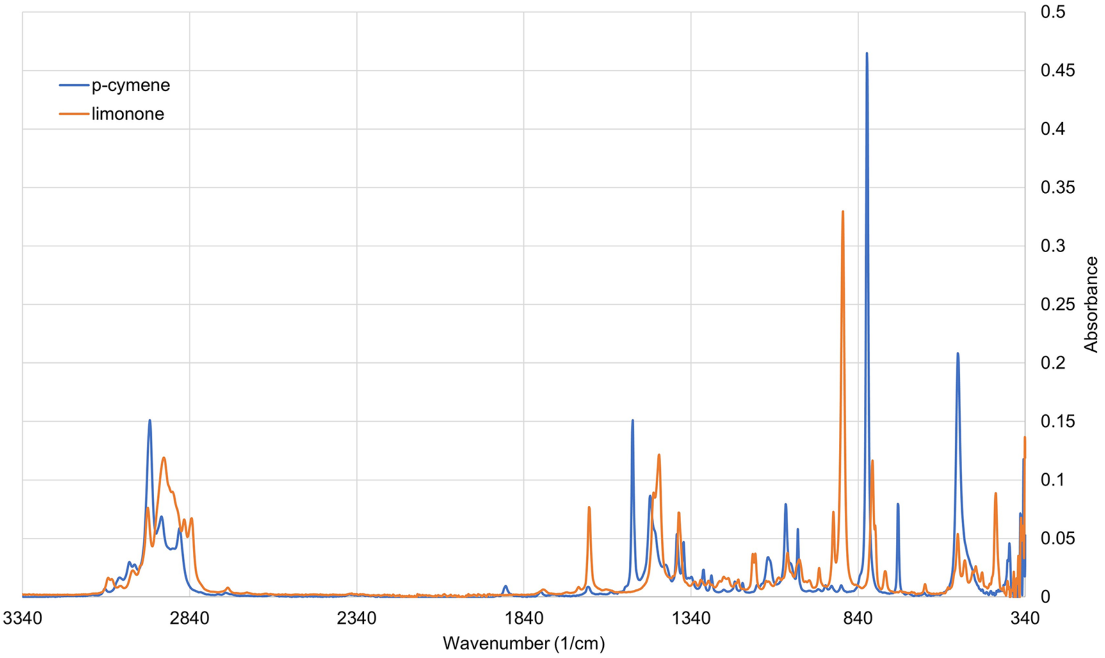


Figure 2. Fourier transform infrared spectra of pure terpenes, *p*-cymene, and limonene.

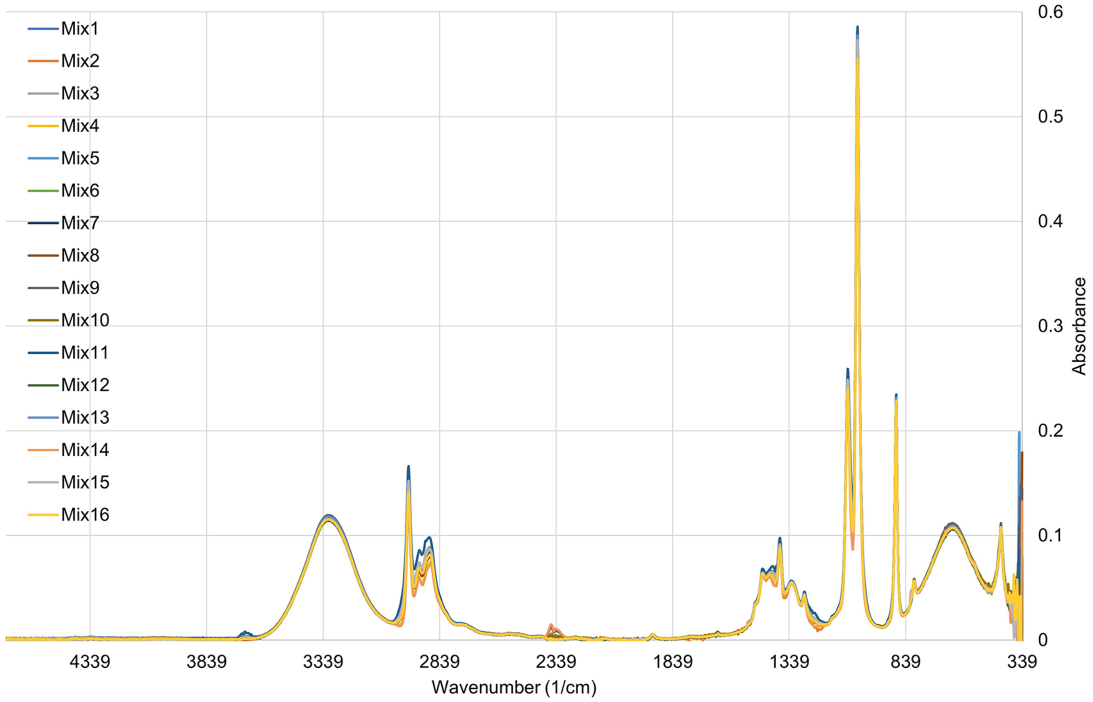


Figure 3. Fourier transform infrared spectra of binary terpene mixtures of *p*-cymene and limonene using a full factorial design ($n = 16$) at the 4700−339 cm^{-1} range.

linearity between the measured and predicted terpene values in the calibration model (Figure 6).

One of the major goals of this study is to identify potential failures and pitfalls of incorrectly following the PLS procedure. As such, we implemented the PLS analysis without any kind of signal processing technique. Results of our analysis showed that using PLS analysis without any kind of signal processing technique using the same wavenumber range (i.e., 1489−718 cm^{-1}) generated higher RMSE and lower R^2 values (Table 1) indicating not favorably good results. The results showcase the importance of applying the signal preprocessing technique prior to any PLS development to provide more accurate multivariate calibration models.

It would be worthwhile to also consider a variety of other signal preprocessing techniques such as a second derivative, standard normal variate, and Savitzky-Golay among others and compare the results presented herein. This extension of the work will provide an in-depth exposure and understanding of how results change due to the use of different signal preprocessing techniques. It should also be noted that, since different mixtures

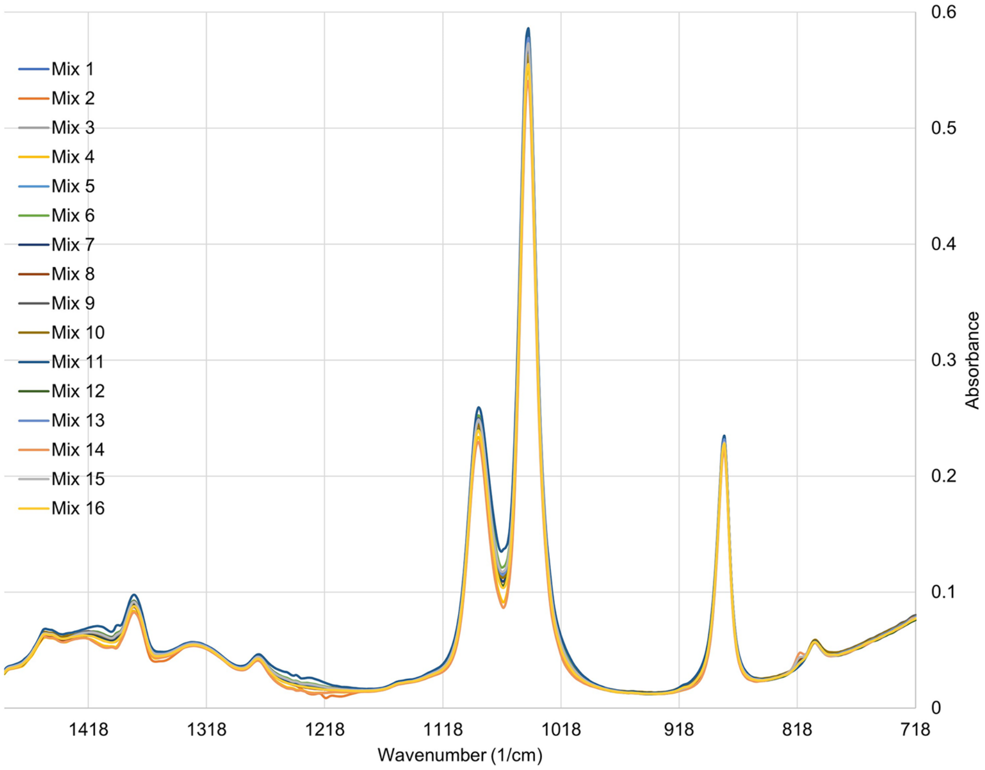


Figure 4. Fourier transform infrared spectra of binary terpene mixtures of *p*-cymene and limonene using a full factorial design ($n = 16$) at 1489−718 cm^{-1}. This spectral range was determined to optimize the results for the cross-validated generated full factorial calibration model.

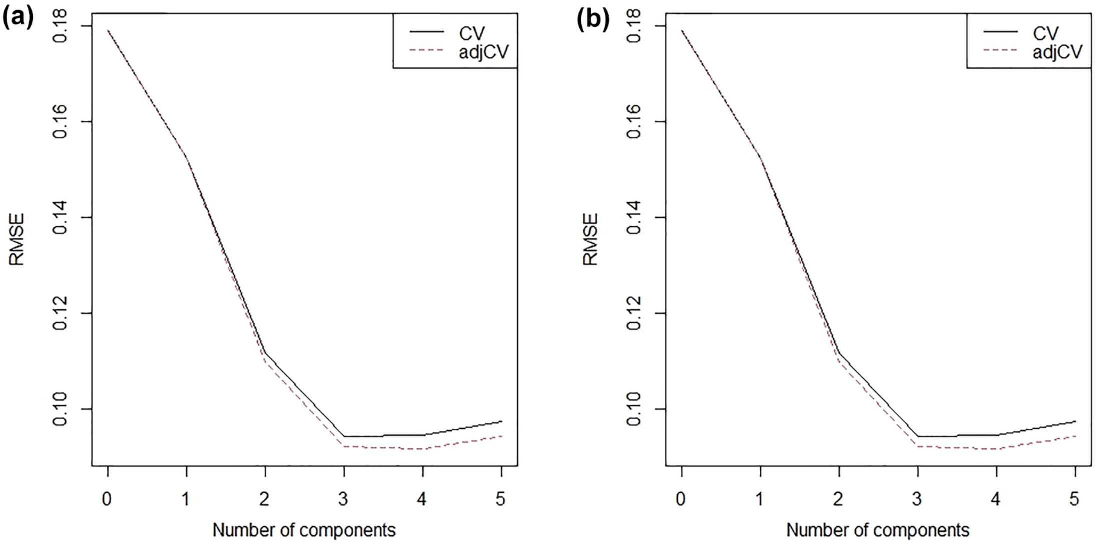


Figure 5. Root mean square error (RMSE) as a function of the number of components for *p*-cymene (a) and limonene (b). As seen in the plot, three components are sufficient to optimize the errors of prediction in the partial least-squares calibration model at 1489−718 cm^{-1}.

were prepared by multiple groups of students, one should also take into consideration the variations in the sample preparation, which could lead to a possible source of error in the generated spectra. As such, sample mixtures prepared by a single and expedient student may yield better results (i.e., higher R^2, lower RMSE) than those prepared by multiple students coming from different groups. Further, PLS typically requires a large number of training samples to build accurate and reliable calibrations.[49]

The ATR-FTIR and chemometric analyses presented herewith are only limited to a training set established using a full factorial design ($n = 16$). This was performed due to the limited availability of time and the number of students needed to perform the analyses. In the future, the activities may also consider an independent test set to assess the performance of the training set.

Implementation

The outlined activity was implemented in the Spring 2023 semester in the instrumental analysis lab course at the University of Scranton, with a total of 20 junior and senior students. The core exercise was designated so that parts I (Preparation of sample mixtures and ATR-FTIR analysis) and II (chemometric

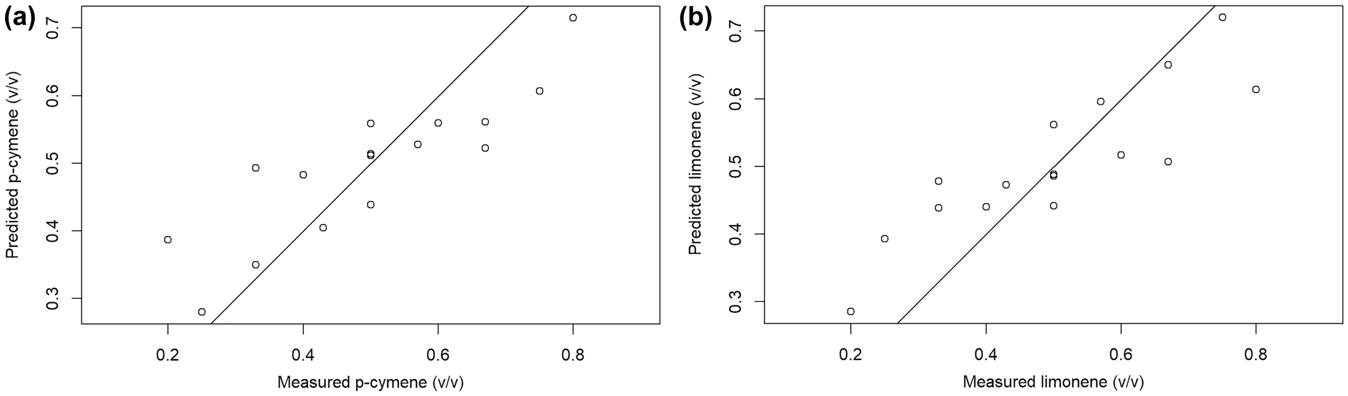


Figure 6. Predicted vs measured (volume/volume) values for *p*-cymene (a) and limonene (b). The plots show moderate linearity at 1489−718 cm^{-1} for *p*-cymene (R^2 = 0.683) and limonene (R^2 = 0.683).

Table 1. Comparison of the root mean square error of prediction (RMSE) and R^2 values for the *p*-cymene and limonene terpene components in binary mixtures using the 1489−718 cm^{-1} spectral range with and without first-derivative signal preprocessing techniques[a]

	With first derivative signal preprocessing		Without first derivative signal preprocessing	
Chemical components	RMSE	R^2	RMSE	R^2
p-cymene	0.0944	0.683	0.123	0.465
Limonene	0.0944	0.683	0.123	0.465

[a] Three components were utilized for all analyses.

analysis of terpene mixtures) could be done and accommodated comfortably each week for each part. That is, each part was carried out in a 6 h laboratory session per week for a total of 12 h in 2 weeks. Student estimates of time required to complete the first part of the activity were consistent with the allocated timeline with most students requiring only 1−2 h to complete the activity (Figure 7). It should be noted that only one FTIR instrument was used, which led to a long waiting time to finish analyses of all mixtures. The second part of the activity, on the other hand, maximized the entire lab period because the instructor performed repeated chemometric analyses for the students to develop full understanding of concepts in PLS (Figure 8). There is a flexibility that the second part of the activity can be finished in only 3 h depending on the depth of the instructor's exercise. The R Markdown code was designed so that students could easily execute the data analysis and observe the output. As students developed a full understanding of each line of the code, they were then asked to make small changes to the code and observe the results.

We have collated the current version of our PLS analyses as an RStudio Markdown code (see Supporting Information). Although our trials integrated this as part of the instrumental analysis laboratory course, instructors could also use this as a standalone laboratory activity and can even be included in analytical chemistry laboratory courses as one of the experiments. The mixture designs can even be modified to include ternary or quaternary mixture components, consequently altering the code to accommodate these changes.

Results of Student Surveys

The prelab survey for part 1 section of the 2-week activity showed that 70% of the students have not used an FTIR instrument before nor do they have prior familiarity with its different parts. There was also a substantial number of students

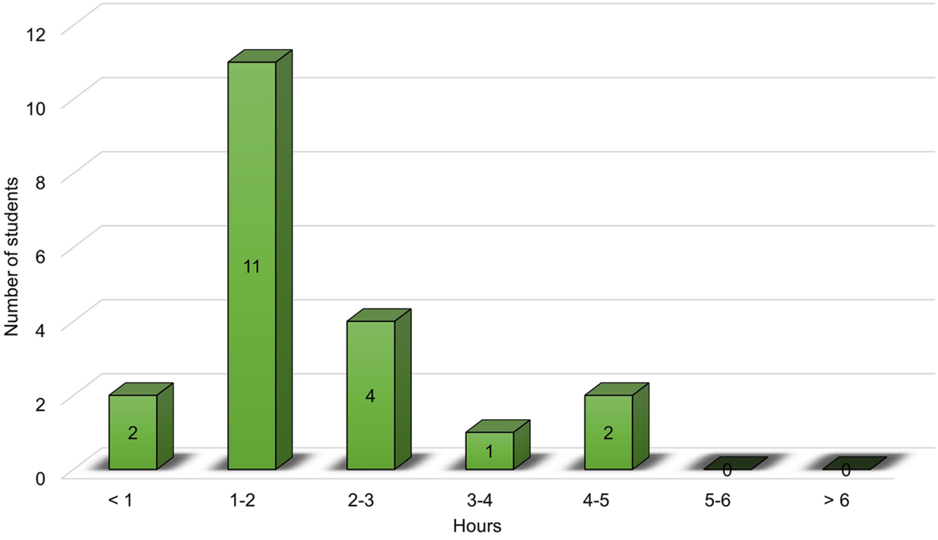


Figure 7. Estimated time frame for students to complete the first activity related to the creation of mixture designs and Fourier transform infrared (FTIR) spectrometric analysis.

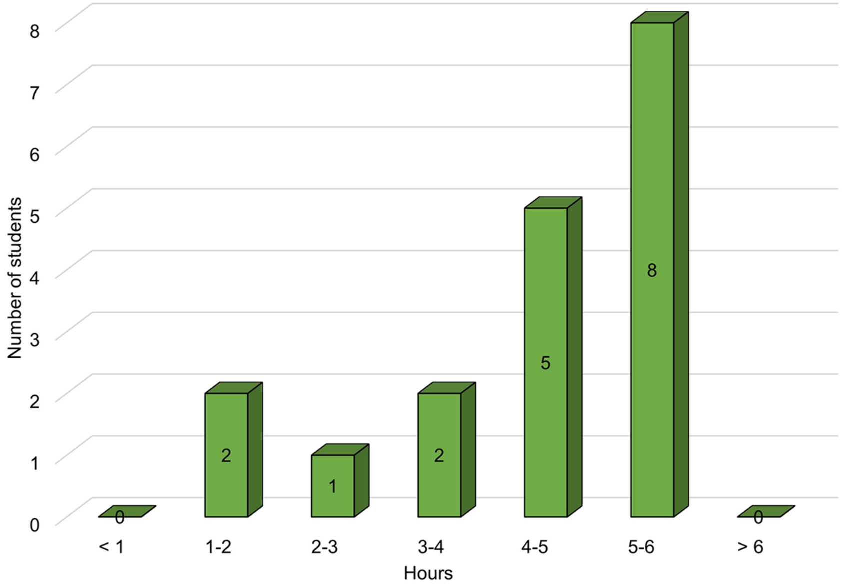


Figure 8. Estimated time for students to finish the second activity related to the chemometric analysis of the data generated in the first activity.

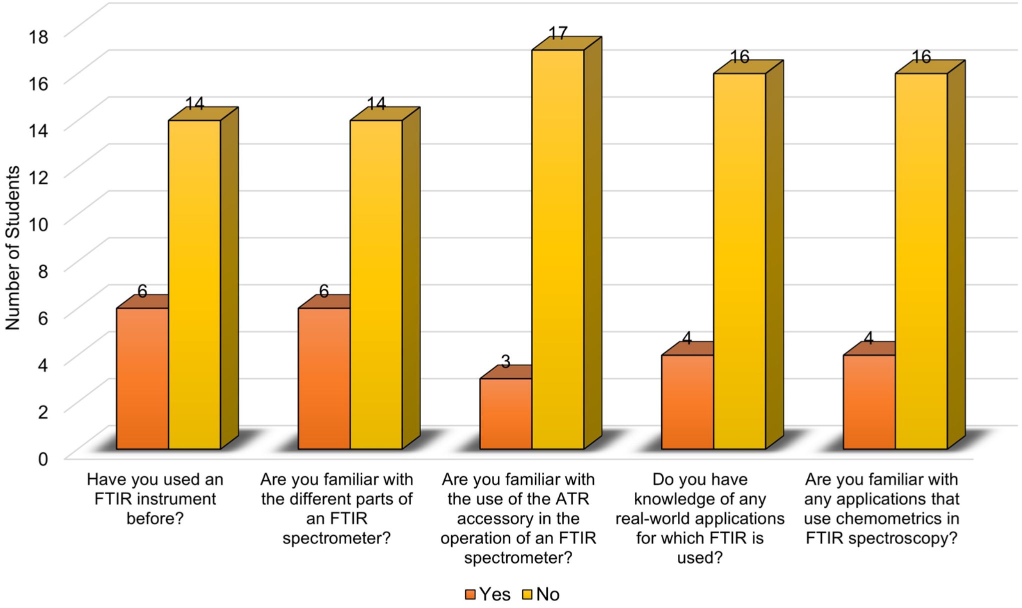


Figure 9. Part 1 (Preparation of sample mixtures and attenuated total reflectance-Fourier transform infrared (ATR-FTIR) analysis) prelab survey.

(80%) who had no knowledge of the real-world applications of the instrument as well as its applications in chemometrics (Figure 9).

After performing part 1 section of the 2-week activity, 100% of the students agreed or strongly agreed that they have gained some basic understanding in the FTIR operations. About 100% of the students also agreed or strongly agreed that they had gained a better understanding of the basic steps in the operation of the instrument (Figure 10). Further, 85% of the students agreed or strongly agreed that they had a clearer understanding of the role and functions of the different parts of the FTIR (Figure 10).

The prelab survey from part 2 of the 2-week activity showed that, although many students (40%) had heard the term chemometrics, only a few were familiar with its chemistry applications (20%). No student was actually aware of the use of chemometrics in any nonchemistry applications outside of their class. Critical to understanding chemometrics is the use of RStudio, and 50% of the class has heard of this programming language. Only 20% of the students had taken any prior computer programming language. Further, 25% had studied, read, or written a piece of R code prior to this class (Figure 11).

The postlab survey for part 2 of the two-week activity indicated that students found the experiment to be an effective introduction to chemometrics using R, with all students agreeing or strongly agreeing that they have gained an understanding on the basic steps in chemometrics using PLS. Many students agreed or strongly agreed (94%) that they identified some of the factors that can affect their chemometric analysis using PLS as well as the possible pitfalls that can occur during data analysis. Many students (83%), on the other hand, agreed or strongly agreed with the idea that they could foresee some applications of PLS in chemistry. The introduction and use of RStudio for PLS analysis was also highly favored by students with 94% of the

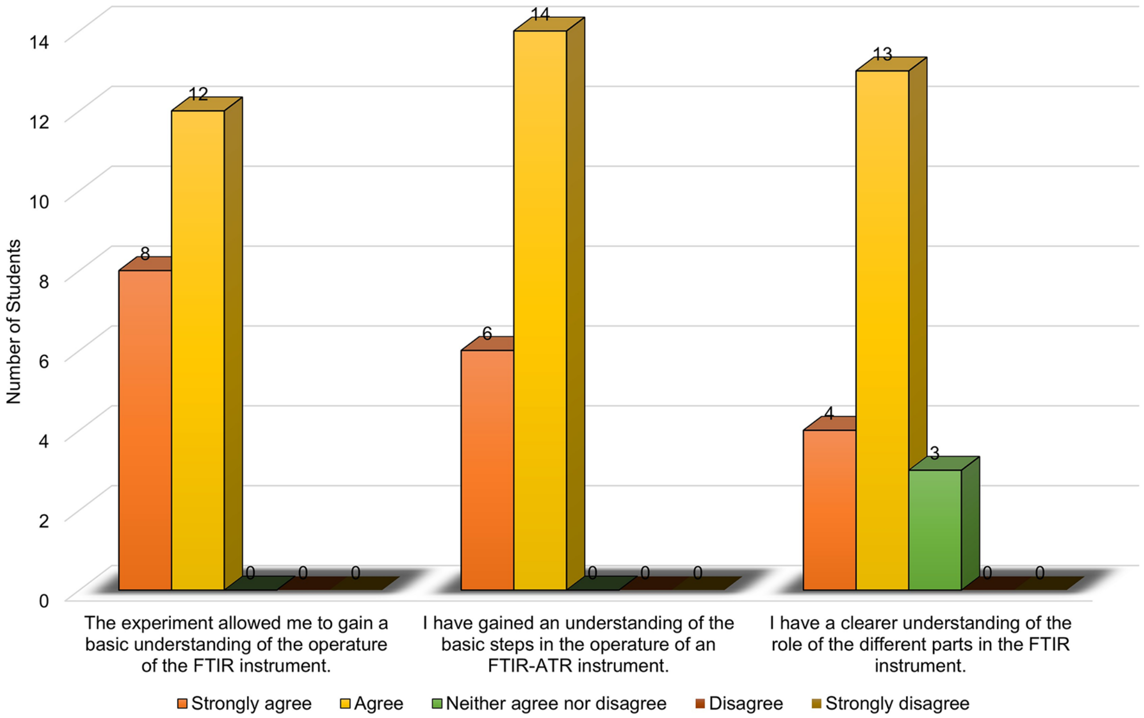


Figure 10. Part 1 (Preparation of sample mixtures and attenuated total reflectance-Fourier transform infrared (ATR-FTIR) analysis) postlab survey.

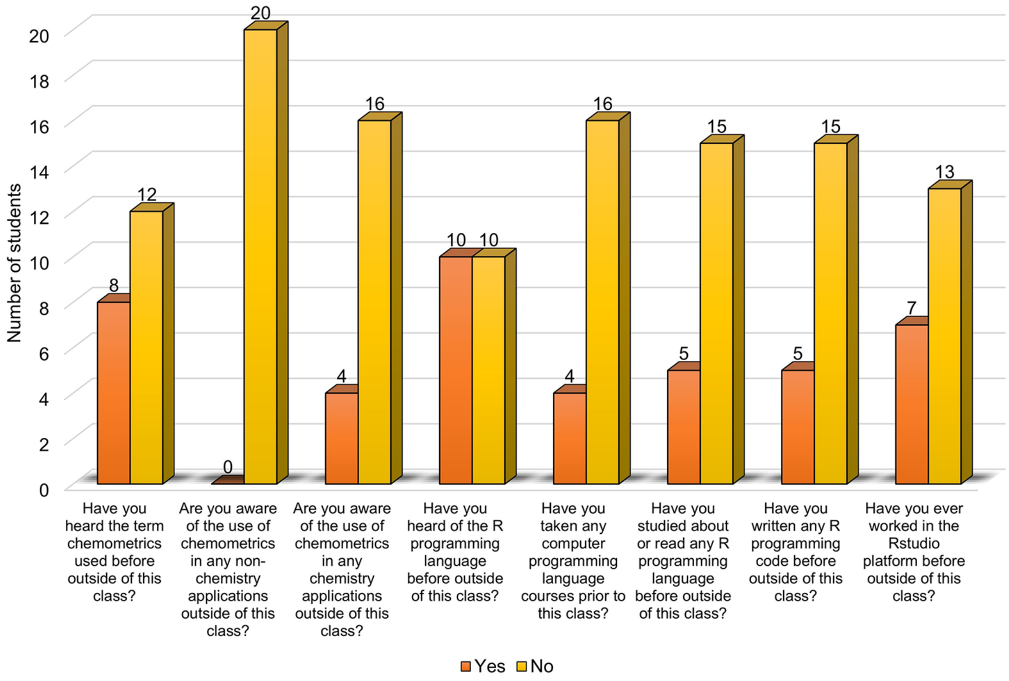


Figure 11. Part 2 (chemometric analysis of terpene mixtures) prelab survey.

students agreeing or strongly agreeing that they were able to read and write simple code using RStudio. Realizing that chemometrics is such a challenging area, it is no surprise that only 44% of the students agreed or strongly agreed that they were interested in learning more about the subject. Finally, the majority of the students agreed or strongly agreed that they enjoyed the exercise (78%) (Figure 12).

■ CONCLUSION

We developed an activity in which students generated mixture designs, performed FTIR analyses, and used the data generated from this analysis for PLS. The main goal of this activity was to develop a PLS calibration model to simultaneously determine the concentrations of *p*-cymene and limonene. The activity, though designed for an instrumental analysis laboratory, can also be part of an analytical chemistry laboratory course. It can also be customized as an independent laboratory activity or modified to allow the analysis of ternary or quaternary mixtures. Further,

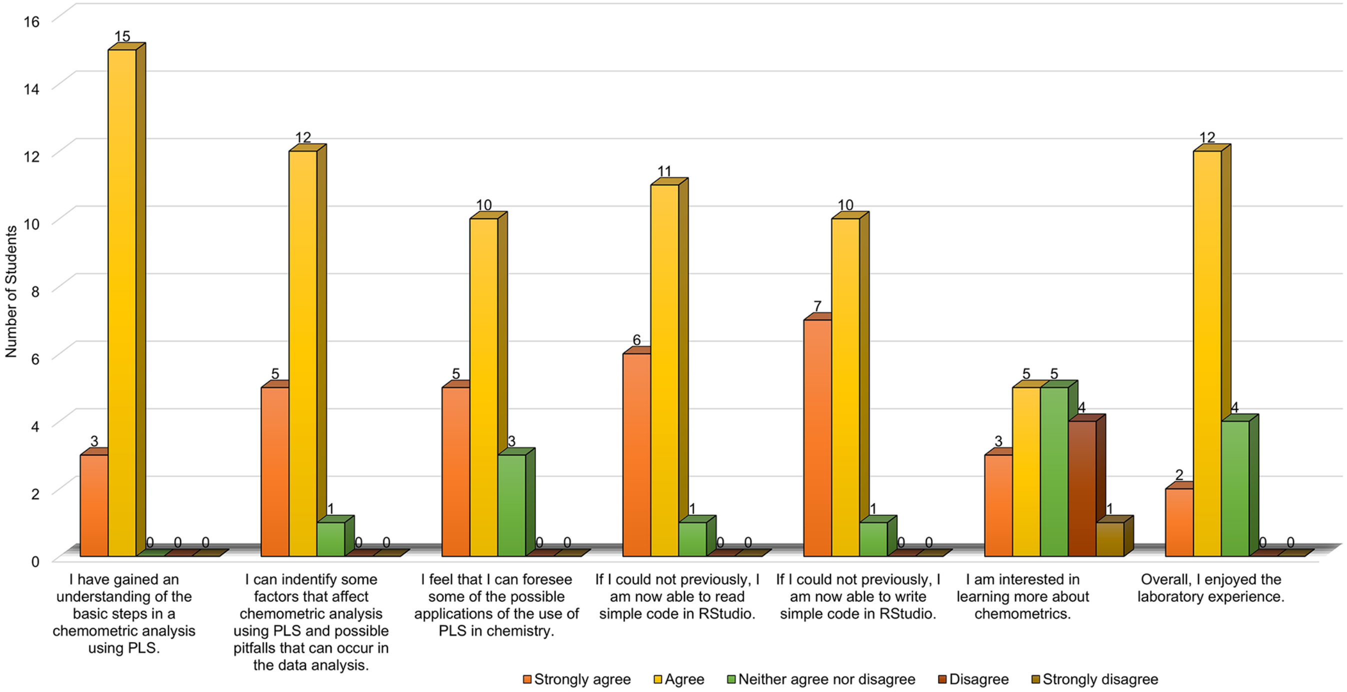


Figure 12. Part 2 (chemometric analysis of terpene mixtures) postlab survey.

the exercise can even serve as a foundation for other independent chemistry-related independent projects. The results of the survey show that the activity was effective in introducing students to the use of FTIR and how to manipulate the data generated for chemometric PLS analysis. This activity will provide an avenue for students to learn the area of chemometrics in light of the growing data generated by various instruments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.3c00358.

Student handout containing background information, step-by-step procedures, and discussion questions for Part 1 (Preparation of sample mixtures and attenuated total reflectance-Fourier transform infrared (ATR-FTIR) analysis) (PDF)

Student handout containing background information, step-by-step procedures, and discussion questions for Part 1 (Preparation of sample mixtures and attenuated total reflectance-Fourier transform infrared (ATR-FTIR) analysis) (DOCX)

Student handout containing background information, step-by-step procedures, and discussion questions for Part 2 (chemometric analysis of terpene mixtures) (PDF)

Student handout containing background information, step-by-step procedures, and discussion questions for Part 2 (chemometric analysis of terpene mixtures) (DOCX)

Chemometric code for the implementation of Part 2 (chemometric analysis of terpene mixtures) using RStudio (http://rpubs.com/obgeneralao/chemometrics) (PDF)

Chemometric code for the implementation of Part 2 (chemometric analysis of terpene mixtures) using RStudio (http://rpubs.com/obgeneralao/chemometrics) (DOCX)

Data set containing the generated FTIR data as well as the mole fraction of terpenes for each mixture. The first column corresponds to the mixture number, the second and third columns correspond to the limonene and *p*-cymene mole fractions, respectively, while the fourth to the last columns correspond to the absorbance values at each specific wavenumber. Figure 3 was generated by plotting the absorbance values across the entire wavenumber range (4700−339 cm^{-1}). The mole fraction of terpenes was calculated using the volumes of the terpenes used in a mixture. For example, limonene in mole fraction in mixture 1 = $\left(\frac{30\ \mu\text{L limonene}}{30\ \mu\text{L limonene} + 30\ \mu\text{L } p\text{-cymene}}\right) = 0.50$. This file was used for Experiment 2: Quantitative determination of binary component terpene mixtures using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy and chemometrics (XLSX)

■ AUTHOR INFORMATION

Corresponding Author

Gerard G. Dumancas − *Department of Chemistry, Loyola Science Center, The University of Scranton, Scranton 18510 Pennsylvania, United States*; orcid.org/0000-0002-8161-4148; Email: gerard.dumancas@scranton.edu

Authors

Noemi Carreto − *Department of Chemistry, Loyola Science Center, The University of Scranton, Scranton 18510 Pennsylvania, United States*

Oliver Generalao − *Center for Informatics, University of San Agustin, Iloilo City 5000, Philippines*

Guoyi Ke − *Department of Mathematics and Physical Sciences, Louisiana State University at Alexandria, Alexandria 71302 Louisiana, United States*

Ghalib Bello − *Department of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, New York 10029, New York, United States*
Arnold Lubguban − *Center for Sustainable Polymers and Department of Chemical Engineering and Technology, Mindanao State University - Iligan Institute of Technology, Iligan City 9200, Philippines*
Roberto Malaluan − *Center for Sustainable Polymers and Department of Chemical Engineering and Technology, Mindanao State University - Iligan Institute of Technology, Iligan City 9200, Philippines*

Complete contact information is available at:
https://pubs.acs.org/10.1021/acs.jchemed.3c00358

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to acknowledge the University of Scranton Department of Chemistry for the ATR-FTIR and the financial resources needed to carry out this research. Dr. Gerard Dumancas would like to acknowledge the University of San Agustin - Center for Informatics for hosting his short-term stint to carry out this research. Dr. Dumancas wishes to thank the Balik Scientist Program of the Department of Science and Technology—Philippine Council for Health, Research, and Development for the opportunity to serve the Filipino community through science, technology, and innovation. The Balik (Filipino word for "Return" or "Repatriate") Scientist Program (BSP) seeks highly trained Filipino scientists, technologists, experts, and professionals residing abroad to return to the Philippines and transfer their expertise to the local community for the acceleration of scientific, agro-industrial, and economic development of the country.

■ REFERENCES

(1) Sun, D.-W. *Infrared Spectroscopy for Food Quality Analysis and Control;* Academic Press, 2009.

(2) Amir, R. M.; Anjum, F. M.; Khan, M. I.; Khan, M. R.; Pasha, I.; Nadeem, M. Application of Fourier Transform Infrared (FTIR) Spectroscopy for the Identification of Wheat Varieties. *J. Food Sci. Technol.* **2013**, *50* (5), 1018−1023.

(3) Kowalczuk, D.; Pitucha, M. Application of FTIR Method for the Assessment of Immobilization of Active Substances in the Matrix of Biomedical Materials. *Materials (Basel)* **2019**, *12* (18), 2972.

(4) Hou, X.; Lv, S.; Chen, Z.; Xiao, F. Applications of Fourier Transform Infrared Spectroscopy Technologies on Asphalt Materials. *Measurement* **2018**, *121*, 304−316.

(5) Simonescu, C. M. Application of FTIR Spectroscopy in Environmental Studies. In *Advanced Aspects of Spectroscopy;* IntechOpen, 2012. DOI: 10.5772/48331.

(6) Schuttlefield, J. D.; Grassian, V. H. ATR−FTIR Spectroscopy in the Undergraduate Chemistry Laboratory. Part I: Fundamentals and Examples. *J. Chem. Educ.* **2008**, *85* (2), 279.

(7) Grassian, V. H.; Schuttlefield, J. D.; Larsen, S. C. ATR−FTIR Spectroscopy in the Undergraduate Chemistry Laboratory. Part II: A Physical Chemistry Laboratory Experiment on Surface Adsorption. *J. Chem. Educ.* **2008**, *85* (2), 282.

(8) Leverette, C. L.; Wills, C.; Perkins, M. A.; Jacobs, S. A. Structural Analysis of Nanofilms Using FTIR Spectroscopy. An Introduction to the Spectroscopic Analysis of Nanostructures for Undergraduate Students. *J. Chem. Educ.* **2009**, *86* (6), 719.

(9) Shepherd, B.; Bellamy, M. K. A Spreadsheet Exercise To Teach the Fourier Transform in FTIR Spectrometry. *J. Chem. Educ.* **2012**, *89* (5), 681−682.

(10) Hanley, Q. S. Fourier Transforms Simplified: Computing an Infrared Spectrum from an Interferogram. *J. Chem. Educ.* **2012**, *89* (3), 391−396.

(11) Zapata, F.; López-Fernández, A.; Ortega-Ojeda, F.; Quintanilla, G.; García-Ruiz, C.; Montalvo, G. Introducing ATR-FTIR Spectroscopy through Analysis of Acetaminophen Drugs: Practical Lessons for Interdisciplinary and Progressive Learning for Undergraduate Students. *J. Chem. Educ.* **2021**, *98* (8), 2675−2686.

(12) Khanmohammadi, M. *Current Applications of Chemometrics;* Nova Science Publishers, Incorporated, 2014.

(13) Maher, C.; Schazmann, B.; Gornushkin, I. B.; Rurack, K.; Gojani, A. B. Exploring an Application of Principal Component Analysis to Laser-Induced Breakdown Spectroscopy of Stainless-Steel Standard Samples as a Research Project. *J. Chem. Educ.* **2021**, *98* (10), 3237−3244.

(14) Sidou, L. F.; Borges, E. M. Teaching Principal Component Analysis Using a Free and Open Source Software Program and Exercises Applying PCA to Real-World Examples. *J. Chem. Educ.* **2020**, *97* (6), 1666−1676.

(15) Yeh, T.-S. Comment on "Identification of Edible Oils by Principal Component Analysis of 1H NMR Spectra. *J. Chem. Educ.* **2019**, *96* (8), 1790−1792.

(16) Cazar, R. A. An Exercise on Chemometrics for a Quantitative Analysis Course. *J. Chem. Educ.* **2003**, *80* (9), 1026.

(17) Rodriguez-Becerra, J.; Caceres-Jensen, L.; Diaz, T.; Druker, S.; Bahamonde Padilla, V.; Pernaa, J.; Aksela, M. Developing Technological Pedagogical Science Knowledge through Educational Computational Chemistry: A Case Study of Pre-Service Chemistry Teachers' Perceptions. *Chem. Educ. Res. Pract.* **2020**, *21* (2), 638−654.

(18) Wheeler, L. B.; Chiu, J. L.; Grisham, C. M. Computational Methods in General Chemistry: Perceptions of Programming, Prior Experience, and Student Outcomes. *Journal of College Science Teaching* **2016**, *45* (3). DOI: 10.2505/4/jcst16_045_03_83

(19) Holme, T. A. Can Today's Chemistry Curriculum Actually Produce Tomorrow's Adaptable Chemist? *J. Chem. Educ.* **2019**, *96* (4), 611−612.

(20) Gilbert, M. K.; Luttrell, R. D.; Stout, D.; Vogt, F. Introducing Chemometrics to the Analytical Curriculum: Combining Theory and Lab Experience. *J. Chem. Educ.* **2008**, *85* (1), 135.

(21) de Oliveira, R. R.; das Neves, L. S.; de Lima, K. M. G. Experimental Design, Near-Infrared Spectroscopy, and Multivariate Calibration: An Advanced Project in a Chemometrics Course. *J. Chem. Educ.* **2012**, *89* (12), 1566−1571.

(22) Balahbib, A.; El Omari, N.; Hachlafi, N. EL.; Lakhdar, F.; El Menyiy, N.; Salhi, N.; Mrabti, H. N.; Bakrim, S.; Zengin, G.; Bouyahya, A. Health Beneficial and Pharmacological Properties of P-Cymene. *Food Chem. Toxicol.* **2021**, *153*, No. 112259.

(23) Alipanah, H.; Farjam, M.; Zarenezhad, E.; Roozitalab, G.; Osanloo, M. Chitosan Nanoparticles Containing Limonene and Limonene-Rich Essential Oils: Potential Phytotherapy Agents for the Treatment of Melanoma and Breast Cancers. *BMC Complementary Medicine and Therapies* **2021**, *21* (1), 186.

(24) Araújo-Filho, H. G. de; dos Santos, J. F.; Carvalho, M. T. B.; Picot, L.; Fruitier-Arnaudin, I.; Groult, H.; Quintans-Júnior, L. J.; Quintans, J. S. S. Anticancer Activity of Limonene: A Systematic Review of Target Signaling Pathways. *Phytotherapy Research* **2021**, *35* (9), 4957−4970.

(25) de Vasconcelos C Braz, J.; de Carvalho, F. O.; de Vasconcelos C Meneses, D.; Calixto, F. A.F.; Santana, H. S.R.; Almeida, I. B.; de Aquino, L. A.G.; de Souza Araujo, A. A.; Serafini, M. R. Mechanism of Action of Limonene in Tumor Cells: A Systematic Review and Meta-Analysis. *Curr. Pharm. Des.* **2021**, *27* (26), 2956−2965.

(26) Ramalho, T. C.; Freitas, M. P.; Cunha, E. F. F. D. *Chemoinformatics: Directions Toward Combating Neglected Diseases;* Bentham Science Publishers, 2012.

(27) Gauglitz, G.; Moore, D. S. *Handbook of Spectroscopy;* John Wiley & Sons, 2014.

(28) Liland, K. H.; Mevik, B.-H.; Wehrens, R.; Hiemstra, P. pls: Partial Least Squares and Principal Component Regression, 2022. https://CRAN.R-project.org/package=pls (accessed 2022-09-28).

(29) R: The R Project for Statistical Computing. https://www.r-project.org/ (accessed 2022-09-14).

(30) Groemping, U.; Amarov, B.; Xu, H. DoE.Base: Full Factorials, Orthogonal Arrays and Base Utilities for DoE Packages, 2021. https://CRAN.R-project.org/package=DoE.base (accessed 2022-03-13).

(31) RStudio Team. *RStudio: Integrated Development for R*, 2020. http://www.rstudio.com/ (accessed 2022-09-14).

(32) Elkamel, A.; Alhumade, H.; Omidbakhsh, N.; Nowruzi, K.; Duever, T. *Chemical Product Formulation Design and Optimization: Methods, Techniques, and Case Studies;* John Wiley & Sons, 2023.

(33) Dutta, S. *Optimization in Chemical Engineering;* Cambridge University Press, 2016.

(34) Kalivas, J. H. Two Data Sets of near Infrared Spectra. *Chemometrics and Intelligent Laboratory Systems* **1997**, *37* (2), 255−259.

(35) Stevens, A.; Ramirez-Lopez, L. *An Introduction to the Prospectr Package*, 2014, https://cran.r-project.org/web/packages/prospectr/vignettes/prospectr.html (accessed 2022-09-14).

(36) Dumancas, G. G.; Ramasahayam, S.; Bello, G.; Hughes, J.; Kramer, R. Chemometric Regression Techniques as Emerging, Powerful Tools in Genetic Association Studies. *TrAC Trends in Analytical Chemistry* **2015**, *74*, 79−88.

(37) Wang, H.-P.; Chen, P.; Dai, J.-W.; Liu, D.; Li, J.-Y.; Xu, Y.-P.; Chu, X.-L. Recent Advances of Chemometric Calibration Methods in Modern Spectroscopy: Algorithms, Strategy, and Related Issues. *TrAC Trends in Analytical Chemistry* **2022**, *153*, No. 116648.

(38) Ríos-Reina, R.; Azcarate, S. M. How Chemometrics Revives the UV-Vis Spectroscopy Applications as an Analytical Sensor for Spectralprint (Nontargeted) Analysis. *Chemosensors* **2023**, *11* (1), 8.

(39) Sadergaski, L. R.; Myhre, K. G.; Delmau, L. H. Multivariate Chemometric Methods and Vis-NIR Spectrophotometry for Monitoring Plutonium-238 Anion Exchange Column Effluent in a Radiochemical Hot Cell. *Talanta Open* **2022**, *5*, No. 100120.

(40) Otto, M. *Chemometrics: Statistics and Computer Application in Analytical Chemistry;* John Wiley & Sons, 2016.

(41) Faber, N. M. Estimating the Uncertainty in Estimates of Root Mean Square Error of Prediction: Application to Determining the Size of an Adequate Test Set in Multivariate Calibration. *Chemometrics and Intelligent Laboratory Systems* **1999**, *49* (1), 79−89.

(42) PubChem. Ethanol. https://pubchem.ncbi.nlm.nih.gov/compound/702 (accessed 2023-04-02).

(43) PubChem. p-cymene. https://pubchem.ncbi.nlm.nih.gov/compound/7463 (accessed 2023-04-02).

(44) PubChem. Limonene. https://pubchem.ncbi.nlm.nih.gov/compound/22311 (accessed 2023-01-20).

(45) Valderrama, A. C. S.; Rojas De, G. C. Traceability of Active Compounds of Essential Oils in Antimicrobial Food Packaging Using a Chemometric Method by ATR-FTIR. *American Journal of Analytical Chemistry* **2017**, *08* (11), 726.

(46) Daferera, D. J.; Tarantilis, P. A.; Polissiou, M. G. Characterization of Essential Oils from Lamiaceae Species by Fourier Transform Raman Spectroscopy. *J. Agric. Food Chem.* **2002**, *50* (20), 5503−5507.

(47) Nakanishi, K.; Solomon, P. H. Infrared Absorption Spectroscopy, Holden-Day, 1977. https://scholar.google.com/scholar_lookup?title=Infrared+absorption+spectroscopy&author=Nakanishi%2C+Ko%CC%84ji&publication_year=1977 (accessed 2023-04-02).

(48) Singh, R.; Das, G. Towards Fluorogenic and Chromogenic Sensing of Heavy Metal Ions in Aqueous Medium: A Mini-Review. In *Advances in Spectroscopy: Molecules to Materials,* Proceedings of NCASMM 2018; Springer, **2019**; Vol. *236*, p 57.

(49) Sanguansat, P. *Principal Component Analysis: Engineering Applications;* Books on Demand, 2012.