World Journal of Chemical Education, 2024, Vol. 12, No. 4, 81-86
Available online at http://pubs.sciepub.com/wjce/12/4/2
Published by Science and Education Publishing
DOI:10.12691/wjce-12-4-2

An Inquiry-Based Experiment Exploring the Theory Behind HPLC Quantification

Shaeen Chetty, Su-Ané Greyling, Letitia Pillay[*]

Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand, Johannesburg, South Africa
*Corresponding author: letitia.pillay@gmail.com

Received September 26, 2024; Revised October 28, 2024; Accepted November 04, 2024

Abstract High-performance liquid chromatography (HPLC) is one of the most used analytical techniques for the identification and quantification of organic molecules in a variety of samples. In this simple laboratory experiment, quantification parameters and the factors affecting them were investigated using organic acids in plant material. Molar absorptivity (ε) and its implications on limit of detection (LoD) and limit of quantification (LoQ) were examined. Students prepared leaf samples by extraction and quantified the malic and fumaric acid concentrations based on calibration curves of a set of standards. Using the data the LoD, LoQ and ε were calculated and the implications of their values tested through a series of structured assessment questions. The learning goals include sample preparation, the application of theoretical calculations on method development, and calculating the organic acid concentrations. The experiment is suitable for senior undergraduate and early postgraduate students in analytical and environmental chemistry courses.

Keywords: *HPLC, organic acids, molar absorptivity, LoD, LoQ*

Cite This Article: Shaeen Chetty, Su-Ane Greyling, and Letitia Pillay, "An Inquiry-Based Experiment Exploring the Theory Behind HPLC Quantification." *World Journal of Chemical Education*, vol. 12, no. 4 (2024): 81-86. doi: 10.12691/wjce-12-4-2.

1. Introduction

High-Performance Liquid Chromatography (HPLC) is a separation technique based on analyte polarity and falls within the section of chromatography and separation [1,2]. In most South African institutions, HPLC is taught at third-year undergraduate level [3,4]. Conventionally, liquid chromatography instrumentation and quantification is taught to undergraduate students with the emphasis on separation, identification and quantification of compounds [5,6]. The application of HPLC can be found in various industries and fields including pharmaceuticals, food and beverage, environmental, biochemistry, and forensic science [7,8]. HPLC is a powerful tool for analysing organic substance however, it can be utilised to analyse metal ions and ionic compounds. Due to the financial strains on most teaching institutions in South Africa, HPLC applications in laboratory experiments are conducted in groups or is taught as theory.

Some of the concepts covered by undergraduate laboratory experiments specifically for HPLC include qualitative aspects (*e.g.* separation, identification of analytes) and quantification (*e.g.* calibration curves, and the limits of detection (LoD) and limits of quantification (LoQ). The latter are crucial parameters in analytical chemistry that provide essential information about the performance of an analytical method, helping to determine its suitability for specific applications and ensuring reliable results [9]. They are calculated using the following equations:

$$LoD = \frac{3\sigma}{s} \text{ and } LoQ = \frac{10\sigma}{s}$$

Where,

- σ is the standard deviation and
- S the slope of the calibration curve

A lesser explored concept is that of molar absorptivity (ε) with HPLC. Generally, undergraduate students are often exposed to this concept when learning about absorption techniques of analysis in their second year of study utilising a UV-Vis spectrophotometer to examine and understand the Beer-Lambert Law [3]. The focus with molar absorptivity (or molar extinction coefficient) at that level, is the light absorption capacity at a specific wavelength and, that compounds have unique molar absorptivity values at different wavelengths [10,11]. They are further introduced to factors that influence molar absorptivity (pH, temperature, structure) with a focus on how conjugation affects molar absorptivity using organic molecule examples to show how increasing the number of conjugated double bonds in a molecule typically leads to a longer wavelength and an increase in absorbance [12]. Students often learn about spectroscopy as the interaction of light with analytes based on Beer-Lambert's law and concentration, as demonstrated in laboratory experiments. However, they may struggle to understand the broader applications of molar absorptivity beyond traditional spectroscopy.

This lab allows them to reinforce their knowledge about separating mixtures using chromatography and quantifying them using spectroscopy. This experiment exploring the calculation and application of molar absorptivity in HPLC is therefore novel in its approach as it demonstrates the connection between chromatography and spectroscopy.

The molar absorptivity for HPLC can be calculated using the equation [13,14]:

$$\varepsilon = \frac{m \times MM \times FR}{0.060 \times l} x\ V$$

Where,

- m is the slope of the calibration curve,
- MM Is the molar mass in g/mol,
- FR is the flow rate in mL/min,
- V is the sample volume in mL,
- 0.06 is a dimensionless constant incorporating the various units (mL, min, ng) to SI units and
- *l* is the path length in cm for the Agilent 1100 (this is specific to each instrument).

This equation is modified from our usual Beer-Lambert Law ($A = \varepsilon lc$) since in HPLC the UV detector is a flow-through cell rather than a static one *e.g.* a spectrophotometer.

Student learning is often easier when they are able to relate to examples and samples/examples have relevance to their daily lives. In this instance, the samples selected are easily identifiable and the analytes two commonly found organic acids. Organic acid content impacts taste, aroma, and preservation of food and beverages [15]. Organic acid analysis is used to ensure product quality and consistency. For instance, it helps assess fruit ripeness or fermentation processes in wine. While there are several techniques for organic acid analysis, HPLC is one of the most commonly used technique [16,17]. It is the preferred method for organic acid analysis due to the separation of analytes, sensitivity, and reproducibility [17,18].

HPLC lab experiments are widely available and there are several articles outlining their use with a wide range of samples and analytes. Typical applications include dyes in candy [19], caffeine and vitamin B6 in energy drinks [20], organic acids in thyme leaf extracts [21], turnips [22] and aflatoxins in wheat [23]. However, in these applications, emphasis tends to be on the analyte and its quantification specifically or more sample preparation using newer extractive techniques such as QuEChERS (quick, easy, cheap, effective, rugged, and safe) [24].

This inquiry-based experiment aims to guide students through quantification of organic acids in leaf samples. By engaging students in sample preparation and data analysis, we enhance their understanding of chromatography and quantification in Analytical Chemistry. It also aims to solidify student understanding of the most fundamental quantification parameters used in Analytical Chemistry. The experiment serves as a constructive platform for extending spectroscopic knowledge into the realm of chromatography. It effectively demonstrates the relevance of analytical chemistry in industrial settings, particularly in ensuring the quality of food and beverages. This experiment stands out by teaching spectroscopy within the context of chromatography and data analysis. It effectively imparts theoretical knowledge of LODs, LOQs, and molar absorptivity while emphasizing their practical application in a cross-disciplinary setting. In addition, the simplicity of the sample preparation and analysis allows for student some hand-on learning with HPLC but the focus more specifically on the nuances of the data. It is versatile, the analytes chosen are commonly found in a range of samples, allowing for flexibility in sample choice. This basic data, together with the theory, provides students with a more integrated understanding of the concepts.

1.1. Learning Goals

This type of analysis is particularly relevant for research areas which quantify organic compounds using HPLC, and its applicability can therefore extend to most branches of chemistry. This experiment is most suitable for third year and Honours level students in the chemistry or environmental chemistry fields. It may also be carried out as one in a series of experiments which involve organic analysis. The laboratory outcomes for this experiment are the following:

1. sample preparation for organic acid analysis,
2. calculating the LoD and LoQ of a method,
3. calculating and understanding the implications of the molar absorptivity in a flow system, and
4. the effect that impurities have in chromatography.

The primary objective is to make students aware of some important criteria to consider when using the HPLC for quantification.

2. Experimental Details

This practical experiment was successfully carried out in senior undergraduate laboratory sessions over a period of four years with the average class size between 30-80 students with lab sessions working in rotation. Typically, groups of between 3-5 students carry out the lab with at least three students sharing data to accommodate their basic statistics. The active lab component runs for between 1.5 and 2 hours after which students were asked to continue working in their groups to assimilate data and discuss the key concepts. The most recent variation of the experiment was run using plant leaf samples, other samples include hard candy and apple juice. Each student was provided with leaf samples which were then crushed and extracted into Millipore or HPLC water. Approximately 0.3 g of sample was accurately weighed into a beaker together with 15 mL of warm water. After ten minutes, the solution was filtered and made up to 25 mL. A further 50 times dilution was made up and a portion transferred to an HPLC vial for analysis.

Calibration data and individual analyte standards were prepared and analysed by the teaching assistant. to allow students time for peer learning at the end of the lab session. Due to the class size, there was insufficient time for students to prepare standards themselves and carry out the analysis in the 3-hour time they are allocated for labs. This experiment could be modified to allow for individual standard preparation and analysis, especially if class sizes are smaller or more time is available. Individual standards (50 ppm) were made up using analytical grade malic and fumaric acids. Additionally, a range of mixed analyte calibration standards ranging from 0 – 100 ppm (0, 1, 10, 50 and 100 ppm) were prepared. For the mobile phase, 3.484 g of K_2HPO_4 was added to 900.00 mL of Millipore water. The pH was adjusted to 2.7 using phosphoric acid and then the solution was made up to 1.00 L. Analysis of all the standards (individual and mixed), and the samples were carried out on an Agilent 1100 set up for optimal separation (Table 1).

Table 1. Operating parameters for the analysis of malic and fumaric acid

Column	C18 (Phenomenex Kinetex)
Wavelength	220 nm
Mobile Phase	20 mM potassium phosphate buffer (pH = 2.7)
Flowrate	1.00 mL/min
Injection Volume	10 µL
Elution	Isocratic (100% mobile phase)
Run time	10 minutes
l (path length)	60 mm ** each instrument will have its own *l* value

During the analysis of the samples, the basic theory of HPLC, an explanation of retention time, separation, resolution, column selection, integration molar absorptivity, and the Beer-Lambert law was explained by teaching assistants. This equipped students with a better understanding of their data and facilitated their interpretation and calculation of molar absorptivity.

Each student was provided with the method details, individual standard chromatograms to allow them to identify the retention times of the analytes, standard chromatograms with (unidentified) analyte areas and sample chromatograms with peak areas for integrated peaks (analyte and other).

3. Results

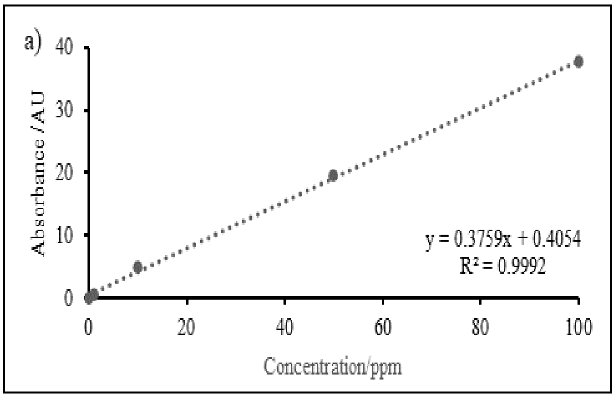
Students used the individual standard retention times to identify the analyte peaks in their standards and in the sample chromatograms. The elution order for the standard mixtures was malic acid (2.5 min) and fumaric acid (5.1 min). Identification of the analytes in the samples involved examining the individual standard chromatograms for matching analyte retention times. The retention time for fumaric acid did not directly match that of the standard (Figure 2A, B). However, analysing the sample spiked with fumaric acid (Figure 2C) allows students to recognise that the sample matrix may have the effect of shifting retention times. It further provides one instance of how to combat/overcome matrix related time shifts. The peak areas for each of their groups analyte standards and samples were then tabulated (Table 2).

The calibration curves (Figure 1) were then derived (using Microsoft EXCEL®) and sample concentrations calculated. Using the LINEST function, students calculated the standard deviation (σ) the curve required for the calculation of LoD and LoQ. The results of these calculations were concentrations for each of their samples, LoD and LoQ and ε values for each of the three analytes (Table 2).

Identifying fumaric acid in the malic acid standard (Figure 2A) underscores the importance of molar absorptivity in assessing LOD, LOQ, and concentration, demonstrating its practical application in analytical chemistry. Students deduce that the likely impurities present in the malic acid standard are fumaric or maleic acid (from the product list provided). Matching the retention time of the impurity to fumaric acid allows for definitive identification. Next, they look at the molar absorptivity values, recognising that ε for fumaric acid at a given concentration is much higher than that of malic acid at that concentration. The implications of higher molar absorptivity on LoD and LoQ values are also explored. Finally, student understanding of how LoD and LoQ should be used in sample preparation is evaluated.

Table 2. Data and calculations for a student group

		Concentration / ppm	Malic Acid Area / mAU.s	Fumaric Acid Area / mAU.s
Data	Standards	0	0.00	1.13
		1	0.46	45.68
		10	4.80	522.99
		50	19.48	2502.16
		100	37.80	5032.70
	Leaf	0.3561 g	34.20	9.20
		0.3239 g	39.90	11.90
		0.2779 g	46.50	9.90
Calculations	Slope		0.3760	50.25
	Intercept		0.4011	2.93
	R^2		0.9996	0.9999
	σ		0.005952	0.1596
	LoD		0.04749	0.009530
	LoQ		0.1583	0.03176
	ε ($M^{-1}cm^{-1}$)		2.5207×10^6	2.9216×10^8
			mg/kg	µg/kg
	Replicate 1		12622	0.4943
	Replicate 2		16218	0.5504
	Replicate 3		22060	0.3144
	Average Concentration		16967 ± 4763	0.4530 ± 0.1233



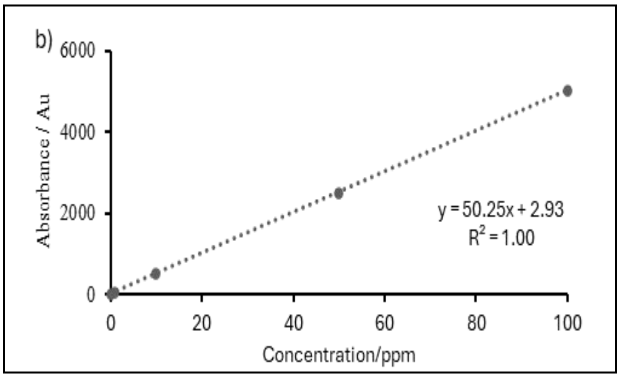


Figure 1. Calibration curves obtained for 0 – 100 ppm a) malic and b) fumaric acid standards

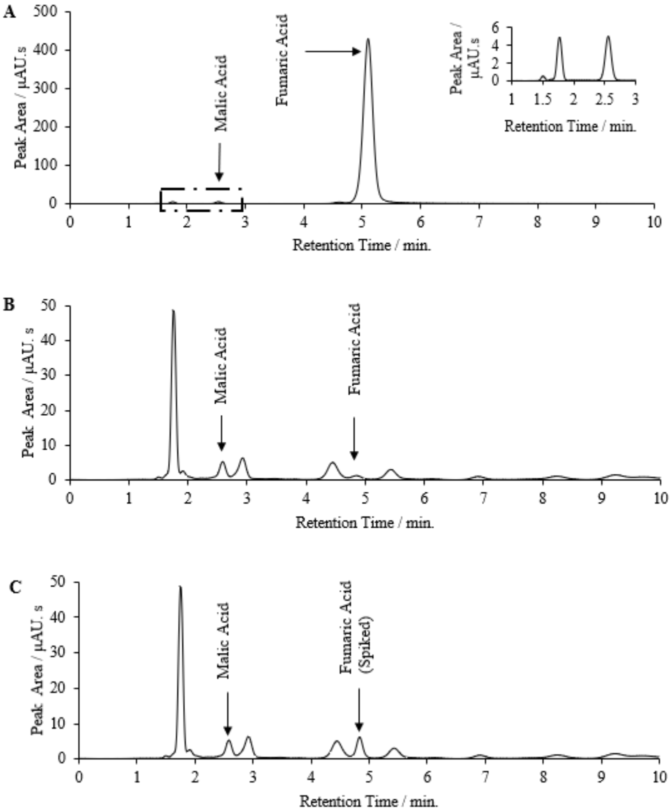


Figure 2. Sample chromatograms for A) 100 ppm standard with inset showing a magnified view of malic acid at 2.54 mins, B) leaf sample and C) a leaf sample spiked with 5 ppm fumaric acid to confirm the fumaric acid peak retention time

4. Discussion

This lab experiment has been performed as part of typical six-week teaching block where student rotation through different experiments teaching different analytical techniques. Each week 3-5 students performed the experiment and at the end of a complete teaching block, each student would have completed the HPLC experiment. A common practice in larger classes was to cycle students through sample preparation to maximize engagement. The laboratory was designed for a two-hour practical investigation and a one-hour interpretation and discussion session. In the practical investigation, students prepared and analysed their respective samples. Standards, single and multi-analyte, were provided to students. Once they obtained their data, students were able to identify and quantify organic acids (malic acid and fumaric acid) in their leaf samples. Students then sat together and completed a worksheet (which follows the basic flow of Table 2) discussing and interpreting their results. To further test their understanding, the following two application questions are included in the worksheet:

- There are several other unidentified peaks in your sample chromatograms. What structural information can you infer about these compounds?
- A candy sample has 21.06 mg/kg of fumaric acid. If the sample was prepared following the practical manual procedure, would you be able to quantify using the current HPLC method?

By initially focusing on the individual components of an HPLC system, students gained a comprehensive, theoretical understanding of their functions [25,26,27]. This foundational knowledge serves as a basis for subsequent learning enabling students to develop well-structured pre-lab use the data to solidify their theoretical grasp of the technique [28,29]. The assessment is structured to progressively deepen student understanding in three parts. The first introduces data processing and interpretation, the next focuses on calibration curves and calculations. This also introduces the student to the concept of data processing and the importance of understanding the data. The last part extends learning beyond basic laboratory exercises, encouraging students to apply theoretical knowledge through collaborative problem-solving. Teaching assistants facilitate discussions and guide students towards solutions. Through analysis of standards, samples, and methods, students explore concepts such as impurities, peak identification, and equation modification. They use social constructivism to determine these answers and are taught skills in collaborative learning [30,31]. Peer collaboration exposes students to different viewpoints, leading to deeper understanding and critical thinking [32,33]. This also increases student motivation and engagement as they feel more connected to the learning process [34].

4.1. Pedagogical Reflection

This experiment was developed for undergraduate students studying Analytical Chemistry, more specifically third-year students. Students are typically introduced to the concept of conjugation in second-year organic chemistry courses. Analytical chemistry courses in the second and third years emphasize chemical analysis and instrumentation, respectively. This constructivist approach enables students to grasp fundamental techniques and apply them to instrumental methods. The experiment was successfully implemented over several years and was successful in improving student understanding of interlinking concepts. The use of relatable samples stimulated student interest and cultivated their awareness of the application of chemistry in industrial and research environments. This provides a good guide for their academic development. The purpose of this experiment was to enable students to master not only the basic principles of chromatography, but to learn the appropriate quantification methods in Analytical Chemistry. Upon completion of the experimental teaching method, components that received positive student feedback (interesting samples, practicing Microsoft EXCEL® for calculations, relevance of molar absorptivity) have been incorporated into subsequent courses. Areas identified as difficult by students (*e.g.* the back calculation example in the worksheet) were revised in lectures.

Analytical chemistry is highly practical, underpinning both industry and research. The experimental aspect involves not only technical proficiency but also cultivating an understanding of its real-world applications and scientific significance. Analytical Chemistry requires rigorous adherence to standardized procedures and since its practical applications often involve precise measurements, students must not only master experimental techniques but also cultivate a rigorous scientific approach to their work.

5. Conclusions

This experiment reinforces the basic skills of sample preparation outlined by other lab experiments, and the use of HPLC for organic analyte analysis. It focuses on the basic principles of compound identification, separation, and quantification. It expands on this knowledge and introduces concepts, molar absorptivity, LoD and LoQ, at an undergraduate level. The setup of the laboratory session is unique in the sense of reinforcing social constructivism. Students complete the sample preparation and analysis, thereafter, discussing and completing the worksheet promoting collaborative learning, facilitated by the teaching assistant. The scaffolding of knowledge is promoted by the teaching assistant who provides them with prepared questions to help students understand the theory and apply it to the laboratory experiment.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the many students who carried out this experiment and the data and feedback obtained.

References

[1] O. Coskun, “Separation techniques: chromatography,” *North. Clin. Istanb.*, vol. 3, no. 2, Art. no. 2, 2016.

[2] C. Horváth, *High-performance liquid chromatography: advances and perspectives*, vol. 2. Elsevier, 2013.

[3] T. J. Wenzel, P. B. Forbes, B. C. Galarreta, B. A. Patel, M. Vogel, and D. K. Wong, “Incorporation of analytical chemistry in the undergraduate curriculum: examples from different regions of the world,” *Anal. Bioanal. Chem.*, pp. 1–10, 2024.

[4] J. F. van Staden, “Analytical chemistry at the University of Pretoria,” *Fresenius J. Anal. Chem.*, vol. 357, pp. 221–223, 1997.

[5] S. P. Perone, J. Pesek, C. Stone, and P. Englert, “Transforming Traditional Sophomore Quant into a Course on Modern Analytical Science,” *J. Chem. Educ.*, vol. 75, no. 11, p. 1444, Nov. 1998.

[6] M. J. Hengel, J. W. Wong, Z. C. Redman, C. Rering, and K. L. Williams, “Analysis of Pesticides in Plant Foods by QuEChERS and Gas Chromatography–Mass Spectrometry: An Undergraduate Laboratory Experiment,” *J. Chem. Educ.*, vol. 97, no. 1, pp. 226–233, Jan. 2020.

[7] P. Gaurav, S. Kushwaha, H. Tiwari, and K. K. Chandrul, “Review on Application of HPLC and UPLC.,” *Int. J. Pharm. Life Sci.*, vol. 15, no. 3, Art. no. 3, 2024.

[8] G. Chawla and K. K. Chaudhary, “A review of HPLC technique covering its pharmaceutical, environmental, forensic, clinical and other applications,” *Int J Pharm Chem Anal*, vol. 6, no. 2, Art. no. 2, 2019.

[9] C. Burgess, *Valid analytical methods and procedures*. Royal Society of Chemistry, 2000.

[10] J. Fabian and H. Hartmann, *Light absorption of organic colorants: theoretical treatment and empirical rules*, vol. 12. Springer Science & Business Media, 2013.

[11] D. A. Skoog, D. M. West, F. J. Holler, and S. R. Crouch, *Fundamentals of Analytical Chemistry*, Tenth edition. Boston, MA: Cengage, 2022.

[12] F. J. Linnig, E. J. Parks, and J. E. Stewart, “Some changes in double-bond structure during the vulcanization of natural rubber,” *J. Res. Natl. Bur. Stand. Sect. Phys. Chem.*, vol. 68A, no. 5, p. 499, Sep. 1964.

[13] M. Locatelli, G. Carlucci, S. Genovese, M. Curini, and F. Epifano, “Use of HPLC in the Determination of the Molar Absorptivity of 4′-Geranyloxyferulic Acid and Boropinic Acid,” *Chromatographia*, vol. 73, no. 9, pp. 889–896, May 2011.

[14] M. Pelillo, M. E. Cuvelier, B. Biguzzi, T. Gallina Toschi, C. Berset, and G. Lercker, “Calculation of the molar absorptivity of polyphenols by using liquid chromatography with diode array detection: the case of carnosic acid,” *J. Chromatogr. A*, vol. 1023, no. 2, pp. 225–229, Jan. 2004.

[15] H. Quitmann, R. Fan, and P. Czermak, “Acidic organic compounds in beverage, food, and feed production,” *Biotechnol.*

Food Feed Addit., pp. 91–141, 2014.

[16] N. Violeta, I. Trandafir, and M. E. Ionica, “HPLC organic acid analysis in different citrus juices under reversed phase conditions,” *Not. Bot. Horti Agrobot. Cluj-Napoca*, vol. 38, no. 1, Art. no. 1, 2010.

[17] I. Mato, S. Suárez-Luque, and J. F. Huidobro, “A review of the analytical methods to determine organic acids in grape juices and wines,” *Food Res. Int.*, vol. 38, no. 10, Art. no. 10, 2005.

[18] A. Robles, M. Fabjanowicz, T. Chmiel, and J. Płotka-Wasylka, “Determination and identification of organic acids in wine samples. Problems and challenges,” *TrAC Trends Anal. Chem.*, vol. 120, p. 115630, 2019.

[19] S. F. Palme, A. H. Johnson, and G. R. Wyllie, “Color, Candy, and Chromatography: A Combined TLC and HPLC Activity for the General Chemistry Laboratory,” *J. Chem. Educ.*, vol. 101, no. 1, Art. no. 1, 2023.

[20] R. E. Leacock, J. J. Stankus, and J. M. Davis, “Simultaneous determination of caffeine and vitamin B6 in energy drinks by high-performance liquid chromatography (HPLC),” *J. Chem. Educ.*, vol. 88, no. 2, Art. no. 2, 2011.

[21] S. C. Purcell *et al.*, “Extraction and antibacterial properties of thyme leaf extracts: authentic practice of green chemistry,” *J. Chem. Educ.*, vol. 93, no. 8, Art. no. 8, 2016.

[22] D. Arias-Carmona, A. Romero-Rodriguez, and M. Vazquez-Oderiz, “Determination of Organic Acids in Brassica Rapa L. Leaves (Turnip Greens and Turnip Tops) Regulated by the Protected Geographical Indication"Grelos De Galicia",” *J. Food Nutr. Res.*, vol. 2, no. 11, pp. 786–791, Oct. 2014.

[23] F. Trombete *et al.*, “Determination of Aflatoxins in Wheat and Wheat by-products Intended for Human Consumption, Marketed in Rio de Janeiro, Brazil,” *J. Food Nutr. Res.*, vol. 2, no. 10, pp. 671–674, Sep. 2014.

[24] A. Shafaei, H. Abbiss, S. Coutts, M. Bannister, J. Bruemmer, and M. C. Boyce, “Tocopherols in seeds and nuts: QuEChERS extraction, HPLC separation, and fluorescence detection,” *J. Chem. Educ.*, vol. 99, no. 5, Art. no. 5, 2022.

[25] D. C. Stone, “Teaching Chromatography Using Virtual Laboratory Exercises,” *J. Chem. Educ.*, vol. 84, no. 9, p. 1488, Sep. 2007.

[26] M. C. Boyce and K. Singh, “Student Learning and Evaluation in Analytical Chemistry Using a Problem-Oriented Approach and Portfolio Assessment,” *J. Chem. Educ.*, vol. 85, no. 12, p. 1633, Dec. 2008.

[27] M. L. Kovarik *et al.*, “Survey of the Undergraduate Analytical Chemistry Curriculum,” *J. Chem. Educ.*, vol. 99, no. 6, pp. 2317–2326, Jun. 2022.

[28] M. K. Seery, H. Y. Agustian, and X. Zhang, “A Framework for Learning in the Chemistry Laboratory,” *Isr. J. Chem.*, vol. 59, no. 6–7, pp. 546–553, 2019.

[29] G. M. Novak, “Just-in-time teaching,” *New Dir. Teach. Learn.*, vol. 2011, no. 128, pp. 63–73, 2011.

[30] A. Udvari-Solner, “Collaborative Learning,” in *Encyclopedia of the Sciences of Learning*, N. M. Seel, Ed., Boston, MA: Springer US, 2012, pp. 631–634.

[31] I. Alzahrani and J. Woollard, “The Role of the Constructivist Learning Theory and Collaborative Learning Environment on Wiki Classroom, and the Relationship between Them,” in *International Conference for e-Learning & Distance Education 2013*, 2013. [Online]. Available: https:// www.learntechlib.org/ p/54801.

[32] R. H. Chen, “Fostering students’ workplace communicative competence and collaborative mindset through an inquiry-based learning design,” *Educ. Sci.*, vol. 11, no. 1, Art. no. 1, 2021.

[33] I. Warsah, R. Morganna, M. Uyun, M. Afandi, and H. Hamengkubuwono, “The impact of collaborative learning on learners’ critical thinking skills,” *Int. J. Instr.*, vol. 14, no. 2, Art. no. 2, 2021.

[34] J.-M. G. Rodriguez, K. H. Hunter, L. J. Scharlott, and N. M. Becker, “A Review of Research on Process Oriented Guided Inquiry Learning: Implications for Research and Practice,” *J. Chem. Educ.*, vol. 97, no. 10, pp. 3506–3520, Oct. 2020.