Didactic sequence to teach laboratory techniques in organic chemistry: analysis of terpenes in common medicines

Secuencia didáctica para enseñar técnicas de laboratorio a la química orgánica: análisis de terpenos en medicamentos comunes

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Resumen

Este trabajo presenta una secuencia didáctica (SD) para enseñar técnicas clave de laboratorio —extracción continua y discontinua, destilación al vacío y a presión atmosférica, extracción por arrastre con vapor y cromatografía en capa delgada (CCD)— a estudiantes de primer año de química orgánica. Basada en el aprendizaje por indagación y una metodología investigativa, la SD integra diversas estrategias educativas: aprendizaje basado en problemas, investigación teórica y experimental, y discusión de resultados. Los experimentos utilizaron medicamentos comerciales para dolor dental y muscular, así como aceites esenciales que contienen terpenos, analizados mediante CCD. Implementada entre 2017 y 2019 en siete sesiones semanales de laboratorio de 3 a 4 horas cada una, dentro de un curso de química orgánica de pregrado en la Universidade Federal Fluminense, Brasil, la SD tuvo como objetivo fomentar el razonamiento deductivo de los estudiantes y la comprensión de las interrelaciones entre las técnicas, vinculándolas con productos medicinales de la vida real. Este enfoque incrementó la participación y el compromiso de los estudiantes, lo que resultó en una mejora en los resultados de aprendizaje. Esta mejora fue evidente tanto en los exámenes finales como en discusiones en clase más activas e informadas en comparación con años anteriores.

Palabras clave: técnicas de laboratorio en química orgánica, secuencia didáctica, cromatografía en capa delgada (CCD), aprendizaje por indagación, educación en química.

Abstract

This work presents a didactic sequence (DS) for teaching key laboratory techniques—continuous and discontinuous extraction, vacuum and atmospheric distillation, steam extraction, and thin-layer chromatography (TLC)—to first-year organic chemistry students. Grounded in inquiry-based learning and investigative methodology, the DS integrates various educational strategies: problem-based learning, theoretical and experimental investigation, and result discussions. The experiments used commercial dental and muscular pain medications and essential oils containing terpenes, analyzed by TLC. Implemented from 2017 to 2019 during seven weekly lab sessions of 3 to 4 hours each in an undergraduate organic chemistry course at Universidade Federal Fluminense, Brazil, the DS aimed to foster students' deductive reasoning and understanding of the techniques' interrelations, linking them to real-life medicinal products. The approach increased student engagement and participation, resulting in improved learning outcomes. This improvement was evident both in final exams and in more active and informed classroom discussions compared to previous years.

Keywords : organic chemistry lab techniques, didactic sequence, thin layer chromatography (TLC), inquiry-based learning, chemistry education.

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Introduction

Terpenes constitute a significant class of natural products, comprising over 30,000 secondary metabolites that fulfill diverse biological roles in various organisms—for instance, acting as defense compounds in plants against viral, bacterial, and parasitic infections. These structurally diverse compounds are found in bacteria, fungi, algae, plants (particularly in essential oils, resins, waxes, and latex), and certain animals (mainly insects and marine organisms) (Xavier et al., 2023). The term *essential oil* dates back to the 16th century, originating from the *Quinta essentia*, a term coined by Paracelsus von Hohenheim of Switzerland, and is linked to their flammability (Dhifi et al., 2016).

Many authors have attempted to define essential oils. According to the 7th edition of the European Pharmacopoeia, they are: "Odorant products with a complex composition, obtained from plant raw extracts, either through steam or water distillation, dry distillation, or an appropriate mechanical method without heating. Generally, a physical method is used to separate the essential oil from the aqueous phase without significantly altering its chemical composition" (El Asbahani et al., 2009).

Several extraction techniques are employed to obtain essential oils, categorized as either classical (hydrodistillation, steam distillation, hydrodiffusion, solvent extraction) or innovative (supercritical fluid extraction, subcritical liquid extraction, solvent-free microwave extraction) (Moghaddam & Mehdizadeh, 2017; Aziz et al., 2018). Gas chromatography–mass spectrometry (GC-MS) is commonly used to identify the constituents of essential oils (Kazemi et al., 2024; Yang et al., 2024). Additionally, terpenes in essential oils can be identified using chromatographic methods such as preparative thin-layer chromatography (TLC) (Kazemi et al., 2024).

Examples of terpenes with medicinal applications include thymol[1], menthol[2], and eugenol[3] (Figure 1). These compounds are valued for their biological activities—anti-inflammatory, antifungal, antibacterial, and analgesic—and are readily obtainable (Kowalczyk, 2020; Best, 2022; Taleuzzaman et al., 2021). Thymol (2-isopropyl-5-methylphenol) is typically extracted from thyme (Kowalczyk, 2020), menthol (5-methyl-2-(propan-2-yl) cyclohexan-1-ol) from mint (Best, 2022), and eugenol (2-methoxy-4-(prop-2-en-1-yl)phenol) from cloves (Taleuzzaman et al., 2021; DeFrancesco, 2021). Structurally, thymol and eugenol are aromatic phenols, while menthol is a cyclohexanol (Dhifi et al., 2016).

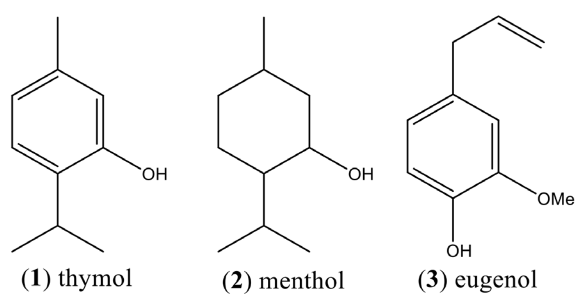

FIGURE 1. Terpenes 1 – 3.

Common laboratory techniques used for separating and analyzing such compounds include continuous and discontinuous extraction, distillation (via rotary evaporator or under atmospheric pressure), and TLC (Nichols, 2019). However, these techniques are often presented in textbooks in isolation, which can hinder students' understanding of their sequential use in producing, purifying, and analyzing organic substances. Integrating these techniques into a coherent framework linked to students' professional interests can enhance learning, particularly through a constructivist, research-based teaching strategy (Ibrahim, 2014).

Essential oils have already been employed to engage students in learning both theoretical and practical laboratory content (Leite, 2020; O'Shea et al., 2012), including TLC, HPLC, and NMR techniques (Bott & Wan, 2013; Pelter et al., 2008; Silva et al., 2009). Terpenes such as thymol, menthol, and eugenol have also been used for teaching NMR, chromatography, and pharmacology (Purcell et al., 2016; Just et al., 2016).

Literature highlights the importance of using experimental sequences (ES) and didactic sequences (DS) to convey that laboratory techniques are not isolated tools, but rather interdependent steps in solving experimental problems. For instance, the analysis of lemon essential oils may involve extraction, evaporation, and TLC; while the fermentation of sugarcane juice requires distillation and titration (Dougherty et al., 1977; Sereda, 2006; Ghirardi et al., 2014).

Chemistry, inherently an experimental science, can be taught using isolated experiments or through ES (García et al., 2024; Joseph, 2000). However, to maximize the teaching-learning process, DS—which integrate various instructional resources beyond experiments—are more effective (Carrizo et al., 2022; Castro et al., 2021; Genoveze et al., 2020; Motokane, 2015). Unlike ES, DS incorporate videos, books, news articles, scientific journals, and other materials to support conceptual understanding.

As supported by the literature and our observations, students overcome learning obstacles more effectively when experiments are presented not as mechanical tasks, but as interconnected activities accompanied by theoretical discussions. A well-designed DS demonstrates how each experiment fits within a larger task framework, fostering critical and reflective thinking.

Didactic sequences can adopt inquiry-based (Jegstad, 2023; Ahmad, 2023) or problem-based learning approaches (Rodríguez-Arteche & Martínez-Aznar, 2016; Caramay & Cruz, 2023), such as confirming the presence of terpenes 1–3 (Figure 1) in commercial pharmaceutical products. These everyday contexts (e.g., medicines for toothache or muscle pain) can serve as motivating themes for DS, integrating ES with diverse educational resources (presentations, videos, papers, news reports, etc.).

In our teaching experience, familiar products like foods, dyes, pharmaceuticals, and cosmetics have proven effective for engaging students. For example, in 2016 we implemented an ES using TLC to analyze food extracts (Fagundes et al., 2016).

This article emphasizes the value of discussing both laboratory techniques and broader scientific issues (e.g., pharmacological effects, technological advances, environmental preservation) to promote meaningful learning and critical thinking (Sjöström & Talanquer, 2014). This work extends our 2016 project (Fagundes et al., 2016) by transitioning from ES to DS, thereby enhancing learning outcomes through the inclusion of diverse pedagogical tools and problem-based approaches.

The proposed DS involved seven weekly laboratory sessions of 3–4 hours each. In addition to mastering laboratory techniques and developing scientific skills, students engaged with theoretical concepts such as hydrogen bonding, tautomerism, resonance, solubility, polarity, and acidity. One key advantage of this approach was its low cost.

Methodology

This study can be characterized as qualitative action research, as it does not aim to analyze results statistically, but rather seeks to foster knowledge construction through the interaction between researchers and participants in the investigated context (Tako & Kameo, 2023).

Didactic Sequence

The didactic sequence implemented in the classroom was structured into four pedagogical moments:

First pedagogical moment/class (inquiry and problem-based learning): this stage began with discussions about students' prior knowledge of laboratory techniques and their applications. A PowerPoint presentation or equivalent instructional material provided by the teacher was used to introduce general laboratory techniques for the separation, purification, and analysis of compounds. To complement this session, a video on thin-layer chromatography (TLC) was shown (Ribeiro et al., 2015).

Following the theoretical presentation, students were shown commercial medicines commonly used for toothache and muscular pain (e.g., plasters) containing thymol[1], menthol[2], and/or eugenol[3]. Students read the package inserts and were then asked to prepare for the second session by writing a brief report (maximum two pages) proposing an experimental sequence to analyze the drug, including the preparation of reference standards through laboratory methods.

The teacher emphasized the use of problem-based learning (PBL), in which students are required to solve a problem relevant to their professional field. Inquiry was thus positioned as a core methodological strategy.

Second pedagogical moment/class (theoretical investigation): students presented their proposed experimental sequences to confirm the presence of terpenes in the pharmaceutical products. These presentations were based on bibliographic research using articles, books, and other sources. The teacher facilitated a discussion among the students, encouraging the exchange of ideas and critical reflection. This phase promoted classroom interaction, as well as the development of competencies and scientific reasoning.

Third pedagogical moment/class (experimental sequence investigation): students carried out the experimental procedures they had proposed. Techniques used included liquid–solid continuous extraction with a Soxhlet apparatus, discontinuous extraction using an Erlenmeyer flask, co-distillation or steam distillation after liquid–liquid extraction, and TLC—following the steps detailed in the experimental section.

Regardless of the specific sequence proposed, all students performed each laboratory technique to allow for comparison and evaluation of the most effective method. At this stage, the teacher's role was to support the development of technical skills and methodological decision-making.

Fourth pedagogical moment/class (discussion of results): students presented their findings orally, supported by written reports. The teacher once again facilitated group discussion, promoting reflection on the experimental process. This moment consolidated knowledge through the development of scientific skills and emphasized the importance of mastering each technique used in the analysis of pharmaceutical products.

Additionally, students engaged in broader, interdisciplinary discussions on topics such as health, society, the chemical and pharmaceutical industries, education, and environmental preservation. This encouraged critical and reflective thinking (Sjöström & Talanquer, 2014). For example, students could reflect on the pharmacological properties of the identified terpenes, preventive healthcare strategies, innovations in essential oil production, or sustainable cultivation of plant species relevant to the manufacture of these substances.

Summary of Classroom Activities:

- First moment: one 3-hour class session.
- Second moment: one 3-hour class session.
- Third moment: four consecutive 3-hour laboratory sessions, one per day. Each session included continuous extraction, solvent evaporation (using simple or fractional distillation and/or a rotary evaporator), and analysis via discontinuous extraction and TLC.
- Fourth moment: oral presentation and discussion of results, supported by a written report.

Table 1 summarizes each pedagogical moment, the didactic resources employed, and the corresponding methodological and educational goals.

Pedagogical Moment	Classroom/ Total time	Pedagogic methodology/ goals	Didactic resource
1	1/3 h	Inquiry and problem-based; classroom discussions; development of students' competence	PowerPoint, package insert
2	1/3 h	Theoretical investigation; classroom discussions/class socialisation; development of students' abilities and competences	Report and oral presentation of the ES analysis proposed by the students based on books, articles, and other bibliographic sources
3	4/3 h	Experimental investigation; development of students' abilities	Experiments, videos, and other educational sources
4	1/3 h	Theoretical investigation; classroom discussions/class socialisation. Development of students' competence and abilities and critical and reflective skills	Report and oral presentation of the ES executed by the students based on class discussions, experimental results, books, articles, and other bibliographic sources

Table 1. Summary of the didactic sequence implemented in the laboratory, indicating the pedagogical moment, didactic resources used, and the educational methodology or objective associated with each session.

Experimental Section

Materials and Reagents

Hexane, ethanol, methanol, ethyl acetate, and dichloromethane were purchased from Vetec. Silica plates for thin-layer chromatography (TLC) (Micro/AL F-254, Silicycle) were used for analysis.

Medicines for toothache (in solution) and for muscular pain (in plaster form) were purchased in local markets; they were selected because their formulations contain eugenol, thymol, and/or menthol. Spices such as thyme, mint, and clove—which contain terpenes 1–3, respectively (see Figure 1)—were also acquired at the market.

For compound detection, a 20% sulfuric acid solution in ethanol, iodine, and UV lamps at 254 nm and 365 nm were employed. The rotary evaporator used for distillation was a Buchi Rotavapor R-114.

Experimental Procedure

Sequence of experiments (Third pedagogical moment; four sessions per week, each lasting 3 to 4 hours):

- **First session:** approximately 20 g of each spice (chosen by the students) was subjected to continuous liquid–solid extraction using a Soxhlet apparatus for 2 hours, with ~170 mL of a suitable solvent (also chosen by the students).

- **Second session:** the extracted solutions were distilled under reduced pressure using a rotary evaporator, or at atmospheric pressure using a conventional distillation apparatus—both methods were selected by the students.

- **Third session:** about 20 g of each spice sample was subjected to co-distillation or steam distillation for 2 hours using ~170 mL of water, followed by liquid–liquid extraction. The solvent used for extraction was proposed by the students.

- **Fourth session:** the spices (20 g each) were subjected to discontinuous extraction for 15 minutes in an Erlenmeyer flask with ~170 mL of an appropriate solvent (chosen by the students).

Following extraction, TLC was performed using the extracts from all procedures: Soxhlet extraction, discontinuous extraction, co-distillation or steam distillation, and a purified thymol sample. The mobile phase (eluent) was proposed by the students.

Chromatographic plates were observed under white light, UV light (254 nm and 365 nm), iodine vapor, and after staining with 20% sulfuric acid in ethanol.

The medicine for toothache, supplied in ethanol solution, did not require further sample preparation for TLC. In contrast, the muscular pain medication, purchased as a plaster, was immersed in ethanol for 15 minutes to extract the organic components prior to TLC analysis.

Hazards

Dichloromethane, hexane, ethyl acetate, methanol, and ethanol are volatile and flammable solvents. All procedures involving these chemicals must be conducted in a fume hood. These substances are harmful if ingested, inhaled, or if they come into contact with skin.

Protective eyewear must be worn when working with UV lamps, as they can damage the eyes and cause skin cancer. Throughout all experimental procedures, students must wear lab coats, gloves, and safety goggles.

Results and Discussion

Feasibility Study of Medicine Analysis

To adapt and evaluate the feasibility of using these medicinal samples in TLC analysis, the experimental procedure was optimized before its implementation in the classroom.

Commercial samples of thymol 1, menthol 2, and eugenol 3 were used as standards in the chromatograms to confirm the feasibility of using essential oil (EO) extracts, and their presence in the medicines was verified through TLC analysis. The toothache medicine, an ethanolic solution, could be directly applied to the TLC plate. However, the plaster required a discontinuous ethanol extraction to solubilize the terpenes of interest. To determine the most suitable solvent mixture as the mobile phase, different ratios of ethyl acetate and hexane were tested with both the toothache medicine and the terpene standards (1–3). A mixture of 25% ethyl acetate in hexane was selected as the mobile phase, as it provided the best separation of the compounds. Figure 2 presents the different mobile phases tested.

The terpene detection on TLC plates was performed using a UV chamber, an iodine chamber, and sulfuric vanillin solution in ethanol (Figure 2).

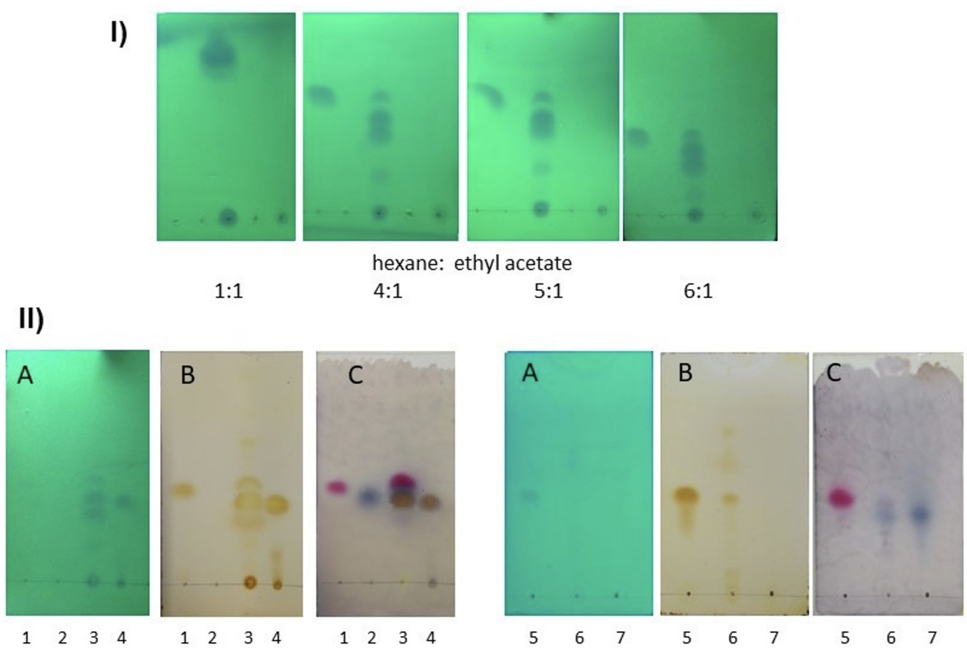

Figure 2. I) TLC visualization under a UV lamp using the toothache medicine and terpene standards to determine the optimal mobile phase. II) TLC of the toothache medicine (spot 3) alongside commercial standards of thymol (spot 1), menthol (spot 2), and eugenol (spot 4). TLC of the plaster sample (spot 6) alongside standards of thymol (spot 5) and menthol (spot 7). Mobile phase: 25% ethyl acetate in hexane. Detection methods: A) UV chamber; B) Iodine chamber; C) Sulfuric vanillin solution.

This preliminary study confirmed the viability of analyzing the active components in these medicines using TLC prior to classroom implementation. Although the medicinal matrices differed, the components were the same, making it possible to use the same eluents for TLC analysis.

Didactic Sequence in the Classroom

Once established, the didactic sequence (DS) involving EO extracts was implemented with 12, 7, 5, 3, and 12 students in five Chemical Engineering classes at the Universidade Federal Fluminense (UFF) during the semesters 2017/2, 2018/1, 2018/2, and 2019/1–2, respectively. The students were divided into groups: six groups of two students in 2017/2 and 2019/2; three groups of two and one individual in 2018/1; two groups of two and one individual in 2018/2; and one group of two and one individual in 2019/1.

The first pedagogical moment occurred during a 3-hour class session. Initial discussions revealed that students had little prior knowledge of laboratory techniques, such as organic compound extraction methods, TLC for drug analysis, or other analytical techniques. A brief tutorial followed, covering continuous and discontinuous extraction, distillation under reduced and atmospheric pressure, steam distillation, and TLC analysis. Students then examined the medicine package inserts to identify whether thymol, menthol, and/or eugenol were listed as ingredients. Based on a theoretical discussion, they were asked to propose a sequence of experiments to analyze these compounds in the medicines and to outline procedures for obtaining terpene standards through spice extraction, distillation, and TLC. Each group could choose to extract one or more terpenes for analysis.

The second pedagogical moment took place a week later in another 3-hour class, where each group presented and discussed their proposed experimental sequences. These involved laboratory techniques for terpene extraction from spices and the use of eluents in TLC analysis. Students proposed techniques such as continuous/discontinuous extraction and steam distillation, along with appropriate solvent systems.

The third pedagogical moment involved the execution of the DS over four weeks, with 3–4 hour sessions. Students successfully produced their TLC standards and selected suitable eluents for analysis. Although reference standards (1–3) were not strictly necessary, extracts containing the major terpenes were used as effective substitutes. Results were reproducible across all classes. Figure 3 illustrates moments from the analysis and TLC results obtained by students.

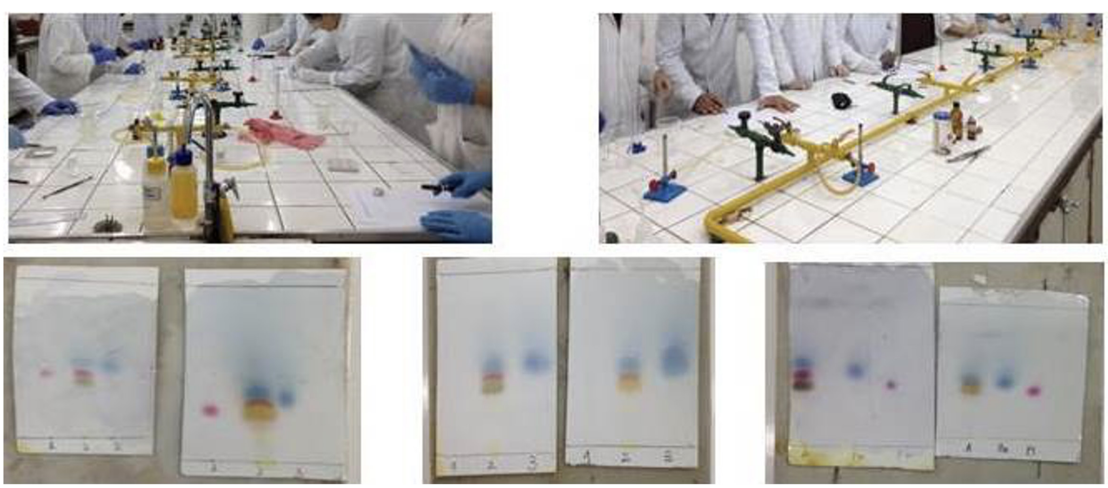

FIGURE 3. Students conducting TLC experiments and their results from the medicine analysis.

The fourth pedagogical moment occurred during a final 3-hour class, where students presented their findings orally and submitted written reports. The class discussion, led by the instructor, showed that students had gained an understanding of laboratory techniques and solvent selection for TLC analysis, effectively identifying the presence or absence of terpenes in the medicine.

Throughout the activities, inquiry-based learning (Jegstad, 2023; Ahmad, 2023) and problem-based learning (Rodríguez-Arteche & Martínez-Aznar, 2016; Caramay & Cruz, 2023) were employed. Social interaction was also encouraged, as suggested by Vygotsky (Marques & Rosa, 2023; Novita et al., 2020), promoting reflection and critical thinking. Discussions included public health, pharmaceutical development, essential oil production, and socio-economic factors affecting medicine access and technological investments.

Perception of Learning Improvement

Improvements in student learning were assessed in three stages:

1. Reports and final group discussions.

2. A final written exam.

3. A final experimental exam.

Reports and oral presentations revealed that students could confirm the presence of terpenes listed in the medicine inserts. Most groups concluded that steam distillation was the most suitable method for obtaining essential oils to serve as standards for the analysis.

The theoretical exam consisted of nine questions on extraction, distillation, and chromatography. Administered without access to reference materials, the average score on the first eight questions was 80%, indicating solid comprehension. An additional question asked students to propose an experimental sequence to analyze curcuminoids or carotenoids in commercial food samples. The average score was 90%, with most students outlining appropriate procedures, confirming their ability to transfer knowledge to new contexts.

1) What techniques can be used to extract organic substances from solid samples?;
2) Describe 2 advantages and 2 disadvantages about the use of Soxhlet extractor and classify this type of extraction according to the method and sample type;
3) Why the rotary evaporator can be used instead of simple or fractional distillation? When to use each one?;
4) What type of experimental method (classify) is TLC considered?;
5) State whether the following statements are true or false and justify your answer. - Only colored samples can be analyzed by TLC. - TLC cannot be used to track a reaction. - The ideal retention factor should be less than 1.;
6) How is the retention factor of a substance calculated in the TLC?;
7) Mention three developers that can be used in the detection of organic substances using TLC. Justify your answer;
8) Which of the statements are correct: - the level of eluent in the chromatography vessel must not reach the point of application of the sample, - the solvent used to solubilize the sample must be the same as the mobile phase, - the substance with the lowest retention factor is which has a higher affinity for the stationary phase.
9) Asked the students to propose an experimental sequence to observe the presence of curcuminoids or carotenoids in commercial food products.

FIGURE 4. Sample questions from the final theoretical exam.

The experimental exam provided further evidence of learning. In a 3-hour session, students independently proposed and executed experiments to detect terpenes in medicines (distinct from those used in class). All groups opted for ethanol-based liquid-solid extraction and used commercial thymol or menthol as reference standards. They also suggested the potential use of thyme or mint for co-distillation to obtain essential oil standards if time permitted. All groups confirmed the presence of terpenes in the samples, further demonstrating effective learning.

The experiment involving the analysis of curcuminoids in foods was proposed as a final practical assessment of students' understanding of organic chemistry. In 2018/2, two groups successfully identified curcuminoids in food samples, confirming the efficacy of the methodology.

Perception of Student Motivation and Methodology Evaluation

After the laboratory sessions, a five-question survey assessed student perceptions of the educational methodology. Questions addressed whether the DS and EO-based sequence made the classes more engaging, increased motivation to learn techniques, improved comprehension of lab methods, and whether the timeline was appropriate.

Approximately 90% of the students agreed that the DS was interesting, the schedule was adequate, the sequence helped them understand laboratory techniques, and it enhanced their motivation to participate actively in class.

Conclusion

It is worth noting that during the course sessions using the didactic sequence, students were required to propose an experimental procedure to analyze the presence of three terpenes—thymol, menthol, and eugenol—in toothache medicines. The muscle pain plaster was used in the final experimental exam. However, this order can be reversed, and other sources of commercial products, such as food samples, can also be employed, either within the didactic sequence or during final assessments.

The experiments can be easily carried out in teaching laboratories. Furthermore, theoretical concepts such as intra- and intermolecular hydrogen bonding, tautomerism, resonance, solubility, and polarity can be effectively addressed using the food-based example presented in this work. This approach fosters the development of students' reflective and critical understanding of laboratory techniques applied to investigative research.

Based on our experience with the implementation of the didactic sequence and the present study, we highlight that the proposed approach contributed to the development of students' critical and reflective thinking on socio-cultural issues, as well as to the acquisition of practical skills and competencies for conducting experimental activities.

Notes

The authors declare no competing financial interest.

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