TEACHING OF THE BASIC CONCEPTS OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) WITH A COLLABORATIVE AND LINKED LEARNING APPROACH: DETERMINATION OF ACROLEIN IN CIGARETTE SMOKE

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Abstract

This article presents a project-based activity, which corresponds to a contextualized (linked) laboratory experience that uses the scientific method performed with students of the undergraduate program "Tecnología en Análisis Químico" (TAQ) at "Universidad Tecnológica de Chile, INACAP" and whose emphasis was to contribute to the theoretical-practical learning of basic concepts of the High Performance Liquid Chromatography (HPLC) using the project based learning (PBL) through the implementation of an analytical method for the determination of chemical substances from "real-life products". In this case, the students participated directly and actively in all the stages that allowed to estimate the concentration of acrolein, a highly toxic compound contained in cigarette smoke, acquiring in the process the fundamental skills of this instrumental analysis technique, which is a tool widely used in the work area of the chemical analyst. Finally, our main objective is to carry out a practical laboratory activity focused on the chemical contextualization in order to promote effective learning, linking students in a broad social context beyond the classroom or laboratory, taking advantage of the intrinsic motivation that a significant activity can generate.

Keywords: Project based learning (PBL), Contextualization, Acrolein, Smoke- cigarette, HPLC.

ENSEÑANZA DE LOS CONCEPTOS BÁSICOS DE LA CROMATOGRAFÍA LÍQUIDA DE ALTA RESOLUCIÓN (HPLC) CON UN ENFOQUE DE APRENDIZAJE BASADO EN PROYECTOS (APB): DETERMINACIÓN DE ACROLEÍNA EN EL HUMO DEL CIGARRILLO

Resumen

Este artículo presenta una actividad basada en proyectos, que corresponde a una experiencia de laboratorio contextualizada (vinculada) y que usa el método científico, la cual fue realizada con estudiantes del programa de pregrado "Tecnología en Análisis Químico" (TAQ) en la Universidad Tecnológica de Chile, INACAP y cuyo énfasis fue contribuir al aprendizaje teórico-práctico de los conceptos básicos de la cromatografía líquida de alto rendimiento (HPLC) utilizando el aprendizaje basado en proyectos (ABP) a través de la implementación de un método analítico para la determinación de sustancias químicas a partir de "productos de la vida real". En este caso, los estudiantes participaron directa y activamente en todas las etapas que permitieron estimar la concentración de acroleína, un compuesto altamente tóxico contenido en el humo del cigarrillo, adquiriendo en el proceso las competencias fundamentales de esta técnica de análisis instrumental, que es una herramienta ampliamente utilizada en el campo laboral del analista químico. Finalmente, nuestro principal objetivo es llevar a cabo una práctica de laboratorio centrada en la contextualización química para promover el aprendizaje efectivo, vinculando a los estudiantes en un amplio contexto social más allá del aula o el laboratorio, aprovechando la motivación intrínseca que puede generar una actividad significativa.

Palabras clave: Aprendizaje basado en proyectos (PBL), Contextualización, Acroleína, humo del cigarrillo, HPLC.

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Educación Química
Volumen 30 | Número 3 | Páginas 15 - 23 | julio 2019
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Introduction

Teaching the technical contents associated to the field of the specialty, in particular those related to the instrumental chemical analysis, represents an interesting educational challenge in which the professors must elaborate strategies for that students can acquire this new knowledge of the integral way.

In this sense, Project-Based Learning (PBL) is a methodology that organizes learning around projects where learning and teaching are done in an active way.

Some of the most recurrent definitions for PBL proposes that: “is a set of tasks based on solving questions or problems through the involvement of the student in research processes in a relatively autonomous way that culminates with a final product presented to others“ (Sanchez, J. M. 2010). Larmer and Mergendoller, (2010), emphasizes that PBL is an effective tool that not only focuses on the contents of a course, but also allows the learning of those so-called 21st century competencies, such as teamwork, self-management, use of technology, critical thinking, etc.

One of the most interesting aspects collected by many authors is to consider that PBL is a learning model in which students work actively in projects that have application in the real-world (Martí et al, 2010; Rodriguez-Sandoval, E. Vargas-Solano, E. M. and Luna-Cortés, J. 2010). It approaches a concrete reality in an academic environment, through the realization that a work project stimulates in the students the development of skills to solve real situations, with which they are motivated to learn; students are enthusiastic about research, discussion of their hypotheses, putting their skills into practice in a real situation. In this experience, the student applies the knowledge acquired in a product aimed at satisfying a social need, which reinforces their values and their commitment to the environment, also using modern and innovative resources (Maldonado Pérez, M. I., 2008).

In this respect, Fergus *et. al*, (2015), proposed that “to consider scenarios of real-life provides a linked with the environment to reinforce scientific concepts and that is not only focuses on the theoretical aspects, but that places students in a broad social context that extends far beyond the classroom or laboratory”. In the same way, indicated that “the contextualization of practical activities in chemistry helps answer one of the main questions of students: Why should I learn something?”

Recently, Parga-Lozano and Piñeros-Carranza (2018), performed an interesting research on the advantages of the contextualization in the chemistry teaching; they exposed that “teachers generally assume the contents in a literal way promoting a teaching focused on reduced disciplinary content”.

Many contextualized didactic activities have been reported for chemical analysis of “real-life” products, where the emphasis is mainly focused on the conceptual learning of chemical analysis techniques and consequently obtain good results (Marcos-Merino, Gallego, and Goméz Ochoa de Alda, 2019; Caamaño, 2018; Leacock, Stancus, Davis, 2011; Carrasquero-Durán, Regalado, Guzmán, and Navas, 2004).

This article presents a project established from the use of the scientific method, but also considers the 8 essential elements of the PBL proposed by Larmer and Mergendoller (2010), to achieve its educational role, these are: 1.Meaningful content, 2. Need to know, 3. A question that direct research, 4. Voice and choice of students, 5. 21st century competencies,

Educación Química
Volumen 30 | Número 3 | Páginas 15 - 23 | julio 2019
DOI: 10.22201/fq.18708404e.2019.3.69368

6. Research with innovation, 7. Elaboration, feedback and revision, and 8. Presentation of results.

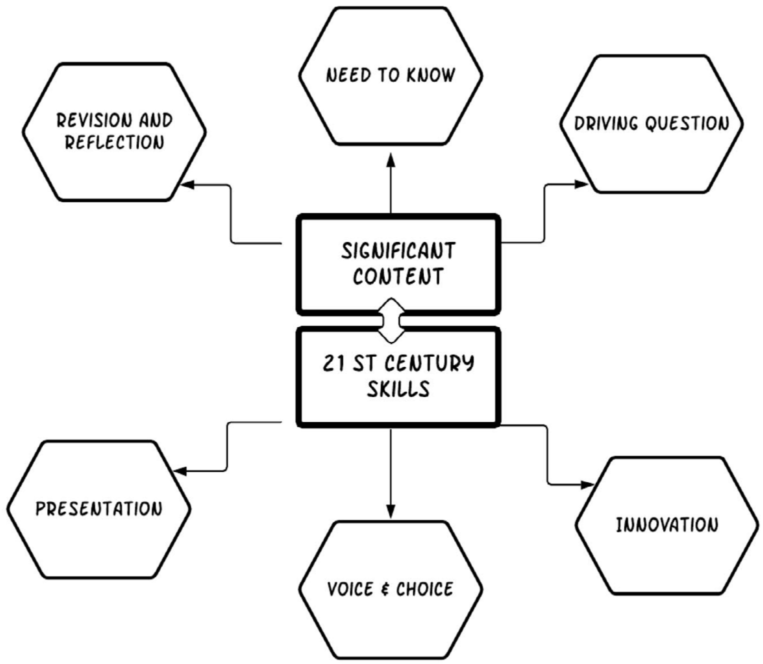


Figure 1. The 8 essential elements for project-based learning proposed by Larmer and Mergendoller and the proposed relationship.

Figure 1 shows a proposal for a relationship between these elements, beginning with the "content" as a central aspect and director of the research. We also consider that the key competences of the 21st century are present in different degrees in all stages of a PBL activity. In this regard, below we present a PBL activity considering the 8 essential elements following an order based on the proposed relationship between them.

Significant Content

This project was carried out with students in the fifth semester course, "Análisis Instrumental II", from the undergraduate program of "Tecnología en Análisis Químico" (TAQ) at "Universidad Tecnológica de Chile, INACAP" whose main objective was the theoretical-practical learning of the basic concepts of the **High Performance Liquid Chromatography (HPLC)** analysis technique where the students participated directly and actively in all the stages that allow the generation of an analytical method for the analysis of chemical substances from "real-life products" and as a consequence to promote meaningful learning. It is expected that at the end of this activity, students will acquire the fundamental skills of this instrumental analysis technique, which is a tool widely used in the work field of the chemical analyst.

Revision and Reflection -Need to know

25 students were separated into groups of 5, they were asked to investigate an "everyday" product related to their daily lives and whose analysis could mean an intrinsic motivation for the HPLC learning process. In this sense, 3 of the 5 groups reported the "cigarette" as an interesting product. From their expositions some important aspects were considered:

Educación Química
Volumen 30 | Número 3 | Páginas 15 - 23 | julio 2019
DOI: 10.22201/fq.18708404e.2019.3.69368

The Pan American Health Organization (PAHO, 2018), reports that, among the countries of America, Chile is the country with the highest prevalence of tobacco consumption in adults and adolescents with 38,7%. In this context, health agencies indicate that more than 100,000 people get sick annually for causes that can be directly attributed to tobacco consumption, resulting in a mortality rate of 54 people per day (IECS-Instituto de Efectividad Clínica y Sanitaria, 2017). Additionally, it is important to consider that 21 of the 25 participating students are active smokers with an average of 7 cigarettes per day.

On the other hand, more than 4000 chemical compounds have been recognized as a result of the tobacco combustion, most of them are toxic and / or carcinogenic (Monoxide and carbon dioxide, ammonia, benzene, benzopyrene, acetone and acrolein, etc.). Due to all of the above, the cigarette can be considered as an interesting product to be analyzed; however, it corresponds to a complex chemical matrix, so it was necessary to delimit the problem.

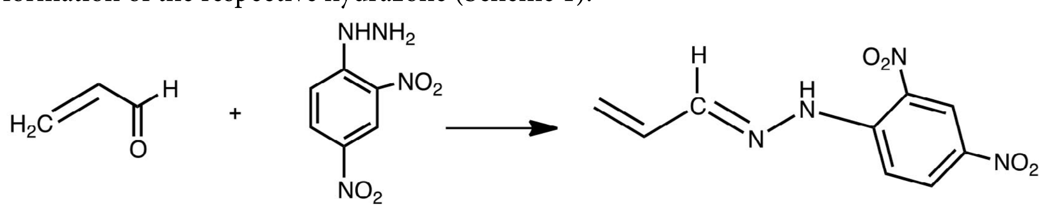
Driving question (delimitation of the problem)

Is there some highly toxic compound that can be analyzed using the HPLC technique? To answer this, two relevant aspects were considered: i) to find a compound present in cigarette smoke that is highly toxic, and has been previously analyzed by HPLC technique and ii) to perform the methodology for isolating the target compound from the cigarette smoke.

Regarding the first point and based on previous research the acrolein was considered as an ideal candidate to be analyzed. Acrolein is an unsaturated aldehyde whose IUPAC name is 2-propenal, which is a colorless or yellow liquid with a sweet smell, highly penetrating, perceptible and highly toxic to humans at low concentrations.

The Department of Health of the United States has stated that: “exposure to acrolein occurs mainly when breathing this substance in the air, product of cigarette smoke and exhaust gases” (Agency for Toxic Substances and Disease Registry, ATSDR, 2011). Recently, Burcham (2017), has published a complete review on the harmful effects of acrolein where was established that there is sufficient evidence that prolonged exposure to acrolein from, specifically, cigarette smoke causes chronic lung damage and that the contact with this chemical even could cause another type of injuries, from cystitis to irreversible damage to the spinal cord.

Acrolein has been determined in a wide variety of samples, such as ambient air, burning combustible gases, etc. (Wright, 2015). Among the most accepted methods for the acrolein determination by HPLC, there are those that use the “typical” reaction of the carbonyl group recognition with 2,4-dinitrophenylhydrazine (2,4-DNPH) for the formation of the respective hydrazone (Scheme 1).



Acrolein

2,4-dinitrophenylhidrazine (2,4-DNPH)

Hydrazone, identificable yellow solution

Scheme 1. Acrolein reaction with 2,4-DNPH to form respective hydrazone quantifiable by HPLC-UV.

Educación Química
Volumen 30 | Número 3 | Páginas 15 - 23 | julio 2019
DOI: 10.22201/fq.18708404e.2019.3.69368

For cigarettes, standard procedures have been developed for the reproducible generation of cigarette smoke samples and the determination of acrolein. The requirements of these are to reproduce the inhalation to understand the basic phenomena of human smoking (Purkis, S. W. *et. al*., 2010).

For acrolein determination, Unfiltered mainstream tobacco smoke is scrubbed of volatile carbonyls by passing each puff through an impinger into a fritted Dreschel trap containing an acidified solution of 2,4-dinitrophenylhydrazine in acetonitrile. An aliquot of the reacted DNPH-smoke extract is syringe-filtered, diluted with 1% Trizma base in aqueous acetonitrile, and subjected to reversed-phase HPLC with UV detection (Wright, 2015).

Innovation

Because the devices of the standardized methods are expensive and difficult to obtain we have innovated the way of collecting and determining the acrolein from cigarette smoke through a methodology arising from creativity and much less expensive considering that the objective of this research is to generate an innovative practical activity that contributes to enhance students´ learning with the available resources of the undergraduate laboratories of our institution.

In this context, Hatchett, (2000), of the School of Chemistry and Biochemistry of the Georgia Institute of Technology, Atlanta, United States, promoted an undergraduate chemistry laboratory activity where students implemented their own analytical module to determine the hydrogen sulfide concentration, H_2S, a highly toxic agent, from cigarette smoke which is the basis for the experimental analytical module used. In this activity the cigarette smoke was received in a glass container with a 0.1M NaOH solution to capture the H_2S, in our experiment this alkaline solution was replaced by 2,4-DNPH solution to receive the acrolein in cigarette smoke.

In Figure 2 a glass bottle containing a 2,4-DNPH solution received the acrolein from the smoke combustion to obtain acrolein-2,4-DNPH complex (hydrazone). On the upper part the cigarette is connected directly through an internal tube with the trapping solution, likewise, from an external side tube connected to the container vacuum was applied with the Gilian Pump allowing an aspiration constant flow.

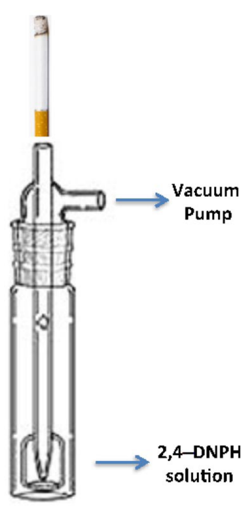


Figure 2. Diagram of cigarette smoke collection used in our practical activity

Educación Química
Volumen 30 | Número 3 | Páginas 15 - 23 | julio 2019
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Experimental Design and PBL

In this section, the students “living” the project of tangible way, that is, transfer the content to the practical.

The acrolein determination was based from the procedures reported literature; each of the steps followed is detailed:

Receiving solution and problem sample. Each laboratory group prepared 30 ml of a solution of 2,4-DNPH, dissolving 30 mg of 2,4-DNPH reactive grade purchased from Sigma Aldrich, 3ml of phosphoric acid analysis grade (p.a) and completed up to 30 ml with the mobile phase. This solution was transferred to the trapping bottle, which received the 5 cigarettes combustion smoke of the well-known brand “Marlboro”, the aspiration of each cigarette, took an average time of 30 seconds, the mixture was allowed to react for 30 minutes. In this procedure the students evaluated the proposed innovation, strengthened the teamwork, self-management, etc., which are relevant aspects of the PBL methodology.

Mobile phase and Calibration curve. The elution of the Acrolein-2,4-DNPH complex is performed isocratically with a mobile phase of Acetonitrile-Water (70:30), both HPLC grade solvents. Five standard solutions of 10 to 50 ppm from the Acrolein-2,4-DNPH standard reagent HPLC grade (25 mg) purchased from Sigma Aldrich, the mobile phase as solvent was used. At this stage the students begin to answer the driving question, they verify that acrolein can be determined by HPLC.

Chromatographic conditions. The analyzes were performed in an HPLC system DIONEX Ultimate 3000 with UV detector, the determination was accomplished in reverse phase using Acclaim 120 column (C18, 120 x 4.6 mm, 5 µm microparticles size), the manual injection loop capacity sample was 20 uL with a mobile phase flow of 1.1 ml / min. Here, they learn in a practical way the basic concepts associated with the HPLC technique.

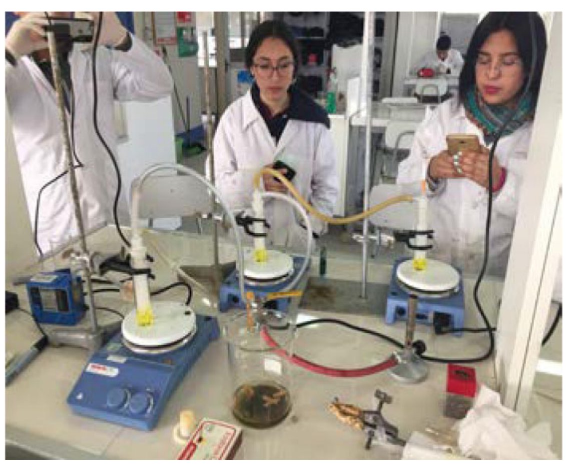

Figure 3. Students collecting acrolein from cigarette smoke

Results

Figure 4 shows the results obtained from the calibration: in a) the concentration (10 to 50 ppm) v/s peak area of the acrolein-2,4-DNPH complexes were plotted. The respective linear equation is: Y(area) = 0,4188X(ppm) + 1.3989 and coefficient R^2 = 0,99146 were

Educación Química
Volumen 30 | Número 3 | Páginas 15 - 23 | julio 2019
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obtained. In b) the chromatogram for the standard concentration of 10 ppm is represented, for the complex a retention time of 3,2 minutes was observed. The sample was prepared for each group from aspiration of 5 cigarettes and respective area values were analyzed at the retention time of 3.2 minutes. The results of the acrolein concentration in cigarette smoke obtained by interpolation are summarized in Table 1. The average concentration was 10,16 ± 1.36 ppm of the complex resulting in 60,94 ± 8,18 µg of acrolein per cigarette. The values of Relative Standard Deviation (RSD) are high to consider an adequate representation of the mean, therefore it is not possible to consider this method as "valid" or compared with those standardized methods.

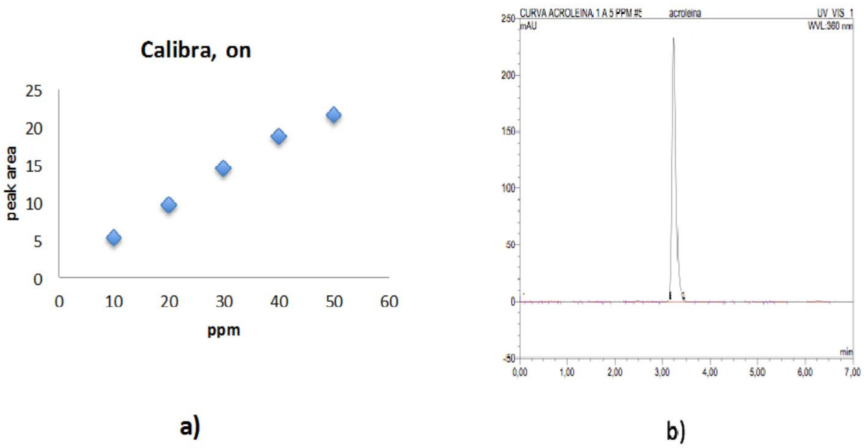

Figure 4. a) Calibration curve, and b) Chromatogram for 10 ppm acrolein complex.

Group	Acrolein concentration, ppm	mg/cigarette
1	11,2	67,0
2	10,9	65,2
3	11,4	68,3
4	8,8	52,8
5	8,6	51,4
Media ($\overline{x}$)	10,2	60,9
(σ_M)	1,2	7,1
(V_p)	(10,2 +/- 1,2) ppm	(60,9 +/- 7,1) ug/cigarette
RDS	11,6	11,6

Table 1. The acrolein-2,4-DNPH complex concentration in 30 ml solution.

Although the results obtained are approach to those accepted methods (58,8 µg/cigarette) (Wrigth, 2015), it is important to consider the acetone- and acrolein-DNPH derivatives co-elute given the current chromatographic conditions. The analysis of acetone- and acrolein-DNPH can only be reported separately when using LC-MS (Foster, 2017) or HPLC-MS determination of acrolein and acetone from $^{13}C_3$ - added to cigarette Tobacco studied by Yip, S. H. (2013). Given the above, there are many improvements that can be applied to make our result more reliable, such as increasing column length, eluting with a gradient, evaluating the temperature effect, etc.

However, the main objective was the implementation of a significant practical activity originated from students' concern, which needed to be addressed in order to contribute to the training of future chemical professionals.

Volumen 30 | Número 3 | Páginas 15 - 23 | julio 2019
DOI: 10.22201/fq.18708404e.2019.3.69368

Conclusions

This activity allowed us to include basic concepts of the HPLC such as retention time, peak identification, stationary and mobile phase, reverse elution, etc. and gave students experience using an HPLC instrument in a meaningful way. It provided with an authentic and relevant project that allowed them to collaborate in teamwork. The PBL became a suitable model of instruction to improve the academic and general achievement students, improving the quality of learning in this area and also contributing in the development of skills for the better information processing, apply learning in novel contexts of problem solving, decision making, planning, contribute to improve the quality of students learning in subject areas such as chromatography.

In this regard, the common consumption of the cigarette and the health concern enhance the learning process through effective link of a real life situation with this experiment. Owing to their familiarity with cigarettes, students were more engaged in and the purpose of the experiment was more clearly defined in their minds, the students reported it was easier to grasp the abstract concepts. Furthermore, co-teaching allowed substantive discussion of scientific method as practiced in laboratory reports and research papers.

Finally, this teaching experience gave us the conviction that the implementation of linked and collaborative experiences helps students to achieve useful and relevant learning, as it connects them with their world outside the classroom, and generates confidence in the potential of their actions, even when the results and methodologies can be improved.

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