Determination of L-Ascorbic Acid in Tomato by Capillary Electrophoresis

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**ABSTRACT:** In an undergraduate bioanalytical instrumental lab, students use capillary electrophoresis to quantify l-ascorbic acid (vitamin C) in food samples. The students are tasked to prepare standards, to obtain the calibration curves, and to determine the quantity of l-ascorbic acid in tomato samples (*Lycopersicon* fruit). A discussion about the function of each component of a capillary electrophoresis instrument, the effect of experimental variables, and the use of different calibration strategies is also given. The experiment has been tested during four courses, showing that lab-activities helped students learn this instrumental technique.

**KEYWORDS:** Second-Year Undergraduate, Analytical Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Electrophoresis, Food Science, Laboratory Equipment/Apparatus, UV–Vis Spectroscopy, Vitamins

Laboratory activities have a relevant role in the science curriculum, but the careful selection of experiments is crucial to encourage student participation and learning. Special attention is particularly required for instrumental experiments as they are time-consuming, expensive, and may not actively engage the student. The solution is an inquiry-type learning model and an adequate analytical method so students can develop abilities and skills such as posing scientifically oriented questions, forming hypotheses, designing and conducting investigations, formulating and revising chemical explanations, and communicating and defending scientific arguments. Only the most useful instrumental methodologies should be included in undergraduate lab-activities to prepare students for their professional life. An example is capillary electrophoresis (CE), whose use has increased in the last decades in industry and research laboratories. Experiments based on CE have been introduced in undergraduate laboratories for separation of drugs, components of soft drinks, ions in water, food preservatives, and benzoic acids. An excellent review of CE methods in undergraduate experiments has been recently published in this Journal. A CE experiment has been designed for the undergraduate laboratory based to the following criteria. First, students will be able to translate the experience to their future professional career, if they understand the analytical protocol. Second, the analysis has to include activities to promote a significant learning of the most important variables and their effect in the electrophoretic separation. Third, student motivation increases when real samples are analyzed, but students must also be reminded of the importance of pretreatment of the sample.

The determination of l-ascorbic acid or vitamin C was selected as an example of instructive experiment. This essential compound is important physiological processes such as the metabolism of iron and the synthesis of collagen. Biotechnological studies have been performed to increase the content of l-ascorbic acid in vegetables and fruits, improving their nutritional proprieties. However, the selection of suitable varieties requires accurate results from hundreds of samples. Moreover, several CE methods have been described for routine determination of l-ascorbic acid in fruit juices, foods, and vegetables using absorbance or electrochemical detection. They have demonstrated that CE is a powerful separation technique that has the advantages of being simple, fast, highly efficient, and selective. Activities based on these procedures are proposed for an undergraduate bioanalytical instrumental laboratory. They include “stop discussion” moments, the investigation of CE separation variables, the use of an internal standard (phthalate) for corrected calibration mode, and the determination of l-ascorbic acid applied in food samples containing tomato (*Lycopersicon* fruits).

**EXPERIMENTAL PROCEDURE**

Protocols

Instrumentation, reagents, and protocols—calibration, sample treatment, CE-separations—are described in Supporting Information that contains instructions for students and instructors. Briefly, students extract l-ascorbic acid from several tomato samples by 2% solution of phosphoric acid. The extracts are centrifuged and microfiltered to eliminate interferences and are transferred to vials with phthalate standard solution for the determination by CE method. Hydrodynamic injection at 1 psi and 5 s is used. Separation is performed at −20 kV and 25 °C with 400 mM borate at pH 8, with 0.01% hexadimethrine bromide as buffer. Analysis with the UV–vis detector allows the identification and quantification of the analyte and the internal standard.

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standard. The calibration curves are obtained from standard solutions prepared by the students.

Participants

The proposed methodology has been tested with second-year students in a bioanalytical instrumental lab. The laboratory experiments were designed for groups of four students, but a smaller group size is recommended. The duration of laboratory session was 3 h. Student experimental data and results from the end-of-course surveys were analyzed for four academic courses (2007–2011).

HAZARDS

Phosphoric acid is skin corrosive, hexadimethrine bromide is harmful if swallowed, and sodium tetraborate shows reproductive toxicity. Students worked with diluted solutions of dangerous reactants, wearing lab coats, protective gloves, and eye protection. Dispense wastes were dispensed in containers, following the guidelines of laboratory hazardous waste management.

RESULTS

The experiment was designed to incorporate different analytical considerations and several teaching–learning experiences. Students were given a laboratory manual including an introduction section as prelab reading, the learning objectives, and the experimental protocol. The initial lab activity of students was the preparation of L-ascorbate and phthalate standards in borate buffer and the measurement of these solutions by UV–vis spectrophotometry. The structures and spectra of the analyte and the internal standard proposed by Galiana-Balaguer et al.18 are shown in Figure 1. The maximum molar extinction coefficient, ε, at 265 nm for ascorbate is 14 500 L cm⁻¹ mol⁻¹ and at 230 nm for phthalate is 7200 L cm⁻¹ mol⁻¹. A discussion was initiated about the detection mode in CE instruments. In case of one-wavelength detection, students understood the importance of choosing the adequate wavelength to register the electropherogram. It is worthy to mention that phthalate shows a low absorbance at the best detection wavelength of L-ascorbate. Nevertheless, phthalate is a suitable internal standard given its properties, such as low-cost, time stability, and similar chemical structure to the analyte (i.e., solubility, electrophoretic mobility). In the case of diode-array detection, the differences between absorption profiles were studied for the ease of identification of CE peaks against the presence of other interferences. Finally, the instructor emphasized the role of buffer solution in the absorption profile as the maximum of ascorbate band is shifted to 256 nm in acidic solution.

A short demonstration of the CE instrument was complemented with specific protocols for using the instrument software. Students were responsible of programming the injection, separation, and detection methods, and controlling the correct data acquisition of standard mixtures. The laboratory manual included questions focused on the fundamental principles of technique and their practical applications. The literature values for the acidity constants and molar mass of both molecules (ascorbic acid, pKₐ = 4.10; pKₐ2 = 11.80; M = 176 g/mol and phthalic acid, pKₐ1 = 2.76; pKₐ2 = 5.41; M = 166 g/mol) were also given in the lab manual. The aim was to promote a discussion about the expected electropherogram from CE separations based on the chemical properties. The students concluded that the mixture is separated according to their charge-to-mass ratio and provides a hypothesis about the elution order. The electrophoretic mobility of internal standard is higher and the students predicted a faster migration toward anode compared to analyte. This order was observed in the electropherogram shown in Figure 2A. The peak identification was experimentally verified comparing with the spectral profile of each molecule using diode-array detection.

The following activity was an oral quiz about the experimental variables and their influence on CE separation. Factors such as injection mode, sample size, voltage, capillary length, temperature, pH, or buffer concentration were discussed. In consultation with the instructor, a hypothetical electropherogram was drawn for each experimental situation. This activity reinforced the students’ knowledge of this specific instrumental technique and stressed the importance of chemical properties in the selection of the suitable analytical method. It also allowed a continuous learning even during the operation intervals of the instrument, improving the efficient use of time.

In the next activity, the standard mixtures with different concentrations of L-ascorbic acid, prepared by students, were injected into the CE to obtain the calibration curves. Although, an automatic processing is possible, it is preferred that students are trained in the acquisition and data treatment. The effect of integration parameters (threshold, minimum peak width, etc.) was evaluated. A discussion was conducted about the selection of peak height (h, in relative units of absorbance) and peak area (A, in units of cm²) as an analytical variable for calibration of ascorbate concentration (c, in units of g/mL). Under described experimental conditions, the calibration curves of students were h = (−239 ± 70) + (58 ± 7)c with R² > 0.991 and A = (−122 ± 50) + (78 ± 7)c with R² > 0.996, respectively. Moreover, the direct calibration was compared to calibration using corrected signals with the internal standard response (phthalate). In about 10% of the separations, the internal standard was necessary to correct the errors during the preparation of solutions by students, that is, filling of flask or during the injection in CE instrument. This comparison showed the benefits of a powerful tool to minimize the experimental errors. In the discussion section of their report, students listed the advantages and limitations of calibrations based on internal standard in routine analysis. The typical figures of merit obtained for different student groups are shown in Table 1. Excellent parameters were obtained in all courses.

![Figure 1. UV–vis spectra of standard solutions (10 mg/L for L-ascorbate and 20 mg/L for phthalate) in 400 mM borate buffer (pH = 8).](image-url)
The next activity was the determination of ascorbic acid in foods with tomato. Application to commercial products helps stimulate student interest and teaches the extra considerations necessary in real-world analysis. The sample pretreatment was based on homogenization, squeezing, centrifugation, and filtration. Phosphoric acid was added to avoid degradation of L-ascorbic acid during the experiment. Students learned about the importance of homogenization and elimination of interferences to get representative and accurate results because the samples are directly injected in the instrument to obtain the result. The influence of food matrix was also evaluated because they applied the strategies to different samples (fruits, juices, and soups) were employed (Figure 2B–F). After the CE separation, the peak identification was based on the migration time of internal standard and analyte and was confirmed by UV spectra registered in the peak (multispectral detector). Students correctly assigned the peaks in the extracted sample electropherograms, even the presence of interferences, because they applied the strategies discussed in the initial laboratory activity. At this point, a continuation of the discussion about the selection of phthalate as internal standard would be beneficial. The instructor can point out that the reliable correction of experimental errors is only achieved due the absence of phthalate in tomato samples, its complete extraction with the proposed treatment protocol, and its close migration time in the electrophoretic separation. The content of ascorbic acid in samples ranged between 0.7 and 34.0 μg/g of sample. The replicates were quite similar with relative errors less than 10%. Eighty-seven percent of student results showed excellent agreement with the values from the instructor’s experiments. Students were specifically asked to use the correct concentration units and to express their results according to the experimental error (significant figures). As part of postlab tasks, students submitted a scientific report on completion of the experiments including several questions in order to evaluate their knowledge about the CE technique and its application.

### STUDENT PERCEPTION

Data collected by observation and survey indicated that the experiment was satisfactorily accepted by students. The student opinion survey was performed for each group in 5 min (18–20 groups by year), including a Likert-scale question and an open-ended question (see the Supporting Information). In the four courses, a majority of the students indicated that the experiment helped them learn the CE technique (83 ± 5% selected agree or very agree options). They also indicated that the experiment was performed with an adequate time distribution (70 ± 10%), difficulty level (81 ± 8%), and dynamic strategy (78 ± 6%). In the open-ended question, the students indicated the experiment stressed the practical aspects in CE separations and the importance of instrumental methods in a routine biotech-laboratory.

### CONCLUSIONS

Teaching experience has to demonstrate the effectiveness in the class, the advantages to achieve learning outcomes, and the key points for their application in other subjects. The proposed experiment has been tested for four years and worked well every time. It illustrates several important concepts and accomplishes several instructional goals of an undergraduate instrumental analysis laboratory. Students consolidate the theory they learned in lectures during the laboratory session. Throughout the activity, students are reminded of the importance of the chemical nature of analytes and how this relates to the analysis (migration time, UV-spectrum). Practical consideration of CE instrument, protocols, and sample application are emphasized. The “stop and discuss” moments allow the utilization of time while waiting for instrument responses. Students discuss and anticipate results, as well as they are able to deduce the capabilities and limitations of the
method. Factors such as the use of an internal standard to correct the experimental errors, the calibration modes, identification strategies, ease of performance, and sample pretreatment are reinforced. Thus, the proposed laboratory experiment based on determination of ascorbic acid in tomato by CE is very useful instructionally.

■ ASSOCIATED CONTENT

4 Supporting Information

Instructions for students: safety and waste disposal, equipment, and experimental protocols; instructions and notes for instructors: safety and waste disposal, equipment, and experimental protocols; student opinion survey. This material is available via the Internet at http://pubs.acs.org.

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Notes

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■ REFERENCES