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Determination of Sodium in Dietary Supplements: An Interdisciplinary Proposal for Teaching Analytical Chemistry in the Technical Chemistry Course – Part I

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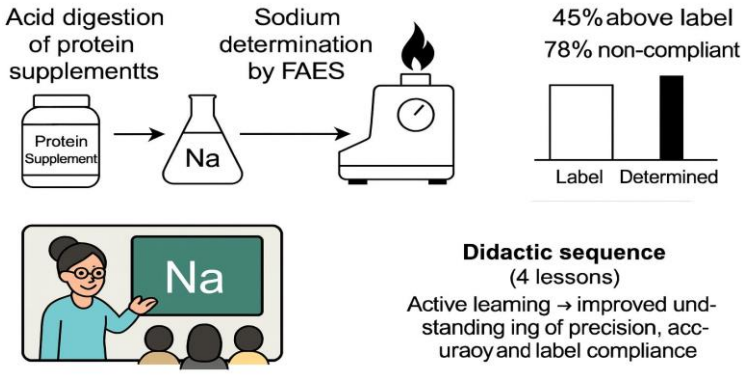
The discipline of analytical chemistry involves developing and improving methods to identify, separate, and quantify substances in a sample. This study proposes an experimental methodology for determining the sodium content of nine (9) protein food supplements using flame atomic emission spectrometry after acid decomposition. Analytical validation focused on precision and accuracy through repeatability, intermediate precision, and analyte addition/recovery tests. The procedure showed adequate precision (RSD < 16.6%) and accuracy (recoveries between 78–110%). Comparison with nutritional labels at a 95% confidence level revealed that 78% of the supplements analyzed had sodium levels statistically different from the declared values, with 45% above the stated values, highlighting the risk of excessive intake. In addition, a didactic sequence was developed, structured in four lessons, integrating analytical chemistry with food and nutrition education. Moreover, the proposed didactic sequence establishes a clear learning flow, guiding students from conceptual discussion to experimental practice and data interpretation. This structure enables them to connect precision and accuracy with real-world analytical problems, enhancing critical thinking and promoting meaningful learning. This active methodology enabled students to connect validation concepts (precision and accuracy) to real-world issues, promoting critical thinking and meaningful learning.

Graphical abstract



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1. Introduction

The discipline of analytical chemistry can contribute to developing critical thinking and understanding phenomena that occur daily in our lives. However, students in technical chemistry courses generally need help understanding some scientific concepts [1]. In addition, analytical chemistry involves several critical mathematical concepts, making it challenging to teach subjects that require a more in-depth mathematical approach, as students generally demonstrate a specific gap in this knowledge [2]. It is worth noting that more teaching materials are needed for technical chemistry courses, as these students often rely on textbooks designed for higher education. Therefore, developing academic resources written in language that is more accessible to this audience is essential.

Therefore, the study was motivated by the need to bring analytical science into the educational context, demonstrating that concepts such as validation, precision, and accuracy are not relevant only to industrial or research laboratories but are also essential for teaching and for the development of qualified chemistry technicians. To date, no studies have been identified that combine sodium quantification in protein supplements with analytical method validation as an interdisciplinary teaching tool.

The validation of analytical methods stands out among the contents covered in analytical chemistry. According to the International Organization for Standardization (ISO) 17025 [3], validation of analytical methods is the confirmation, by testing and presenting objective evidence, that specific requirements are met for a given intended use. In other words, analytical validation must demonstrate, through experimental studies, that the method meets the requirements of analytical applications and that the results are reliable [3].

Unreliable analytical data can lead to disastrous decisions and irreparable financial losses [4]. To achieve this, certain validation parameters must be evaluated, namely: linearity, linear working range, sensitivity, robustness, precision, accuracy, limit of detection (LD) and quantification (LQ), selectivity and specificity. Among these, accuracy and precision will be discussed in this article.

1.1 Precision and accuracy

Precision and accuracy are important requirements in method validation, as they enable estimation of errors and variations in analytical results. The method validation procedure must include steps necessary to demonstrate that the results obtained are reliable and reproducible [5].

The precision of a method refers to the degree of agreement among independent test results obtained under specified conditions. Precision is expressed as the relative standard deviation (RSD%) or the coefficient of variation (CV%). This criterion can be assessed using repeatability, intermediate precision, and reproducibility. Repeatability is defined as the result of independent tests using the same method, on the same test material, in the same laboratory, performed by the same operator and using the same equipment on the same day [6, 7].

Intermediate precision, on the other hand, refers to precision assessed using the same sample, identical samples, or standards, with the same method and laboratory, but under different conditions, such as analysis days, analysts, equipment, and environmental conditions. Reproducibility is assessed by verifying method performance through interlaboratory comparisons. However, since it requires collaboration among different laboratories, it becomes more difficult to evaluate [6, 7].

Accuracy refers to the degree of conformity between the result of a measurement and a reference value. To determine this criterion, analyte fortification/recovery tests and the use of certified reference materials (CRM) are most commonly employed. Analyte fortification and recovery tests are related to accuracy because they reflect the amount of a given analyte recovered during the process relative to the actual amount present in the sample. Certified reference materials are characterized by a methodologically valid procedure for one or more specified properties and are accompanied by a certificate that provides the value of the selected property and its associated uncertainty. However, due to the high cost of CRMs, their use is often limited [8].

Figure 1 illustrates the different levels of precision and accuracy achievable for an analytical result, depending on the experimental method adopted. The archer represents the experimental method, and the arrows' positions on the target determine the results. Depending on how the archer shoots the arrows, different outcomes on the target are possible. The closer the arrows land to each other, the greater the precision. Accuracy, on the other hand, is higher when the arrows hit the center of the target. Target 1 shows low precision and low accuracy, as the arrows are scattered and far from the center. Target 2 has low precision and high accuracy because the arrows are far apart, yet their average is close to the center. Target 3 displays high precision and low accuracy, because the arrows are close to each other but far from the center. Finally, Target 4 illustrates the result that every experimental method aims to achieve: high precision and high accuracy – that is, results that are closely grouped and near the reference value.

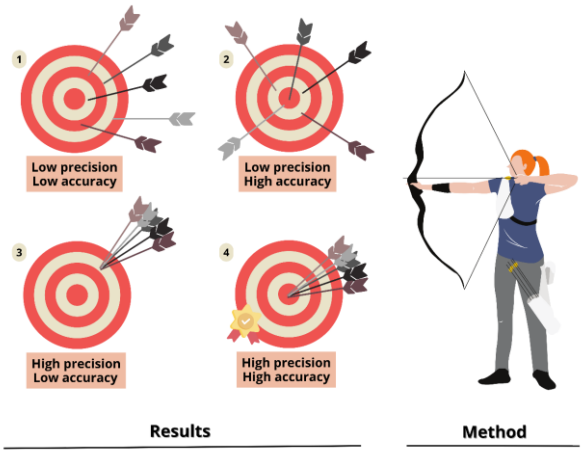

Fig. 1. Didactic representation of the different combinations of precision and accuracy in experimental results, illustrated through archery targets.

1.2 Evaluation of supplements and the consequences of sodium intake

Sodium quantification in dietary supplements represents an opportunity to link analytical chemistry with health and nutrition education. Law No. 13.666/2018 amends Article 26 of the Law of Guidelines and Bases of National Education (LDB), establishing the inclusion of food and nutrition education among the cross-cutting themes of the school

curriculum. In this context, teaching analytical chemistry by determining sodium levels in protein dietary supplements can be a practical approach to stimulate student interest and connect chemical knowledge with their everyday realities, making learning more meaningful [9].

In recent decades, the commercialization and consumption of protein dietary supplements have increased significantly, especially among individuals who engage in physical exercise and seek muscle gain. It is worth noting that these products are commonly used by students and young adults, who, in most cases, consume them indiscriminately [10].

It is well known that adequate intake of micronutrients and minerals (such as sodium) is extremely important for the practice of physical activity. Furthermore, sodium is the main cation in extracellular fluid and plays essential roles in acid-base balance, water balance, and neural function. In addition, its deficiency may lead to muscle cramps, mental apathy, and reduced appetite [11]. However, excessive intake of sodium is associated with the development of non-communicable chronic diseases (NCDs) such as high blood pressure, kidney disease, and cardiovascular disorders [12]. To date, no studies have been identified that combine sodium quantification in protein supplements with analytical method validation in an interdisciplinary educational context. Considering these aspects, the present study aimed to develop and apply an experimental methodology for the determination of sodium in protein food supplements, integrating concepts of analytical method validation with food and nutrition education to enhance the teaching-learning process in technical chemistry courses.

2. Material and Methods

2.1 Instrumentation

Flame atomic emission spectrometry (Flame Photometer – FAES, 910M, Analyser, Brazil) was used to determine sodium (Na, λ = 589 nm) in protein food supplement samples. The vials and glassware used in FAES analyses, and the acid decomposition of the samples were previously washed with distilled water, immersed in a 10% v v^{-1} nitric acid (HNO_3) solution for 24 h, and rinsed with distilled water. For the acid decomposition of the samples, 65% v v^{-1} nitric acid (HNO_3) (PróQuímios, Brazil) and 30% m m^{-1} hydrogen peroxide (H_2O_2) (Pró-Químios, Brazil) were used. A hotplate (TMA20R, Thelga, Brazil) and borosilicate tubes were used for heating in a water bath. For recovery tests, sodium chloride (NaCl) PA (ACS Científica, Brazil) and a mono elemental sodium (Na) standard solution 100 mg L^{-1} (Analyser, Brazil) were used. All solutions were prepared with appropriate dilution with distilled water. Polypropylene tubes with a capacity of 50.0 mL were used for storage.

2.2 Sample preparation

Nine samples of protein food supplements of different brands and flavors were purchased. A mass of approximately 0.5000 g of each sample was weighed and transferred to the decomposition tubes (in authentic triplicates for all samples), and after that, 3.00 mL of HNO_3 65% v v^{-1} was added to the tubes. The mixture was placed in a water bath at 95 °C for 40 minutes. After that, 2.00 mL of H_2O_2 30% m m^{-1} was added to the mixture, leaving it to decompose for 40 minutes. Once the decomposition was complete, the samples were filtered using Whatman No. 1 filter paper (11 µm nominal pore size), and subsequently the filtrate was diluted to 25.0 mL with distilled water. FAES analyzed sodium.

2.3 Analytical validation

The precision of the proposed procedure was evaluated through repeatability and intermediate precision. This work did not perform reproducibility because it requires collaboration between different laboratories.

2.3.1 Precision: Repeatability

The repeatability evaluation was performed using the relative standard deviation (RSD) of the sodium concentration of the authentic triplicates of each of the 9 dietary supplement samples. The same operator was used on the same day, with the same equipment and laboratory for this test. To calculate the RSD, equation 01 was used:

$$RSD = \frac{S}{Xm} \times 100 \quad (1)$$

Which Xm is the arithmetic mean, and S is the absolute standard deviation.

2.3.2 Intermediate Precision

RSD also evaluated intermediate precision. However, the agreement between the sodium concentrations of 4 randomly selected samples (S3, S4, S5, and S7) was compared. These samples were analyzed on different days using different operators. Equation 1 was used to calculate the RSD.

2.3.3 Accuracy: Analyte Addition and Recovery

The accuracy of the procedure was evaluated by analyte addition and recovery tests. Sample S2, which was randomly selected from the analyzed samples, was used to assess this analytical parameter, adding the monoelemental standard solution of Na 100 mg L^{-1} and the solid standard of NaCl (previously dried in an oven for 1 h at 120 °C). For this purpose, 2.5 mL (100 mg L^{-1}) of the monoelemental sodium standard solution was added to 0.5000 g of sample S2 to obtain a final concentration of 10 mg L^{-1}. In addition, 0.0025 g of the solid NaCl standard was added to 0.5000 g of a new sample S2 to obtain a final concentration of 39 mg L^{-1}, as shown in Figure 2.

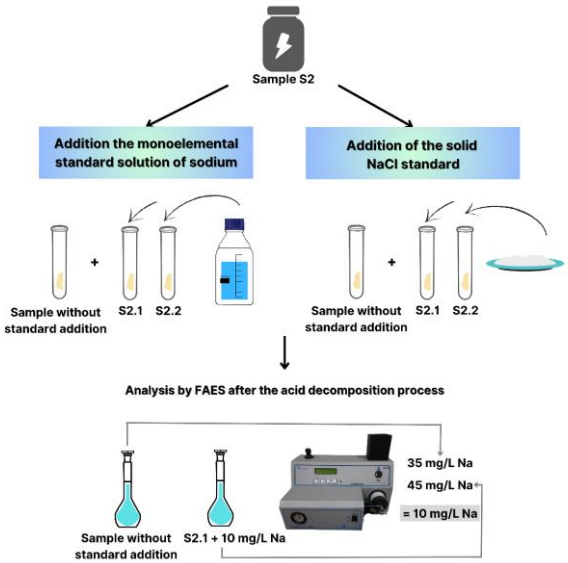

Fig. 2. Schematic illustrating the Analyte Addition and Recovery procedure.

The additions were performed to compare the analyte's recovery percentages using liquid or solid standard solutions. A low final concentration of Na (10 mg L^{-1}) and an intermediate concentration of Na was added to test the recovery in different equipment working ranges. Since the addition and recovery test requires a large volume of monoelemental sodium standard, solid NaCl was used to prepare the intermediate concentration (39 mg L^{-1}).

Finally, with and without adding the analyte, the samples were subjected to the same acid decomposition procedure and FAES quantified sodium. The addition and recovery tests were performed in authentic duplicates. The didactic scheme with the steps of the analytical procedure is presented in Figure 3.

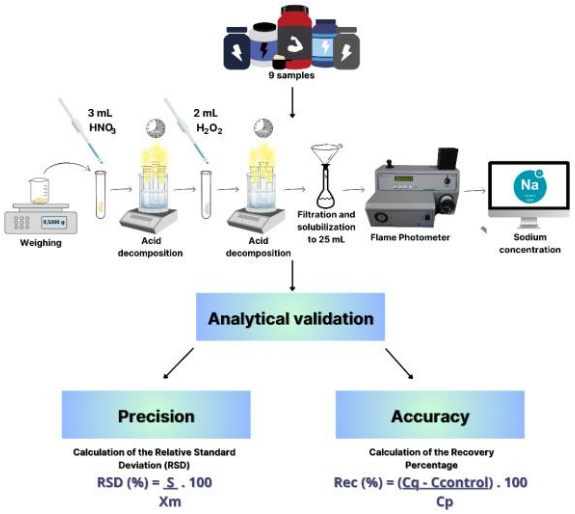

Fig. 3. Flowchart with the methodology used.

Equation 2 was used to calculate the recovery percentage.

$$Recovery\ (\%) = \frac{(Cq - Ccontrol)}{Cp} \times 100 \qquad (2)$$

Cq is the sample's concentration value with the added standard, $C_{control}$ is the control concentration value (without the addition of standard), and Cp is the concentration value of the added standard.

2.4 Compliance with Label

After obtaining the sodium concentrations of all samples, compliance with the manufacturer's label was assessed. The comparison of concentrations was assessed using a student's t-test with a 95% confidence level.

2.5 Educational Research Method

This study adopted an educational research perspective to structure and evaluate the proposed didactic sequence. The methodological approach followed the dominant stages typically employed in educational research, beginning with the identification of a learning problem related to students' difficulties in understanding analytical validation concepts. Based on this diagnosis, an instructional intervention was designed as a four-lesson didactic sequence integrating analytical chemistry with food and nutrition education. Instructional materials and experimental activities were developed in alignment with the technical chemistry curriculum, and the sequence was subsequently implemented with students in a real teaching context. Learning outcomes were analyzed by considering students' participation, the quality of their experimental reports, and their ability to interpret precision, accuracy, and label compliance. This set of procedures characterizes an applied, qualitative, and descriptive educational research framework, in which the effectiveness of the didactic sequence is assessed through evidence of student engagement, conceptual understanding, and the development of critical thinking throughout the intervention.

3. Results and Discussion

3.1 Analyte

The term "analyte" is given to the substance that is of interest to be studied in a sample. According to the quantity of analyte present in the sample, it can be classified as major, minor, trace, or ultra-trace. For an analyte to be classified as major, its concentration in the sample must be between 1% and 100%. The analyte classified as minor is present at concentrations ranging from 100 mg L^{-1} (100 ppm) to 1% of the total sample. When it presents a concentration range of 1 µg L^{-1} (1 ppb) to 100 mg L^{-1} (100 ppm), it is considered trace, and for a concentration lower than 1 µg L^{-1} (1 ppb), it is called ultra-trace. For samples in the solid physical state, the ranges are the same, but in 1 kg of the sample (i.e., mg kg^{-1} for ppm and µg kg^{-1} for ppb).

The analyte/sample concentration ratio directly affects the reliability of the results, since lower analyte concentrations increase the probability of experimental deviations. As previously described, precision assesses the degree of dispersion in analyte results obtained from multiple analyses of the same sample. Resolution No. 166 of July 24, 2017, of the National Health Surveillance Agency (Anvisa) [5], which establishes validation of analytical methods, does not set a maximum acceptable RSD value for precision; however, it discusses acceptance criteria. According to the resolution, the RSD must be determined and justified based on the method's objective, the method's intrinsic variability, the working concentration, and the analyte concentration in the sample [5]. Therefore, the maximum acceptable RSD value depends on the method used and the analyte type and concentration in the sample [13].

This is also demonstrated by the Horwitz Trumpet (Fig. 4), which shows the relationship between method precision and analyte concentration in the sample for reproducibility tests. As observed in Fig. 4, the RSD increases as the analyte concentration decreases. When the analyte corresponds to a concentration greater than or equal to 1% (i.e., majority) in relation to the sample, the acceptable RSD value is approximately 2%. When the analyte concentration is between 100 mg L^{-1} and 1% m v^{-1} of the sample (i.e., minority), the maximum acceptable RSD is approximately 5%. For analytes in trace concentrations (1 µg L^{-1} to 100 mg L^{-1}) the RSD can reach 50%. Finally, for analytes at ultra-trace concentrations (≤ 1 µg L^{-1}), the RSD can exceed 50% (Figure 5) [14, 15].

Although the Horwitz Trumpet is used for interlaboratory testing (i.e., to assess reproducibility), the relationship between analyte concentration and the RSD it expresses can also be used to verify repeatability and intermediate precision. It is worth noting that some studies have also accepted higher RSD values in more complex analyses, mainly due to difficulties during sample preparation [15].

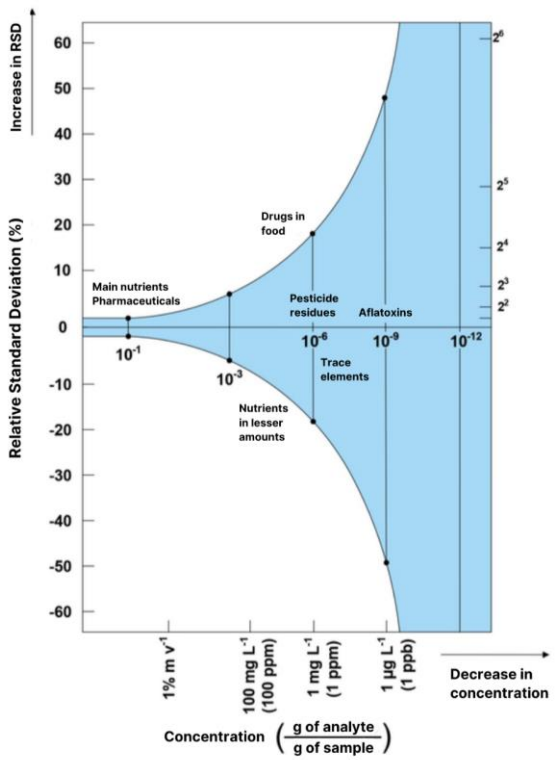

Fig. 4. Representation of Horwitz trumpet, adapted from Harris, D.C. [16].

Table 1 presents the precision values (repeatability and intermediate precision) for the 9 protein dietary supplements, calculated as relative standard deviations (RSDs). It is worth emphasizing that, after acid decomposition, the filtrate from all samples was solubilized, resulting in analyte concentrations between 1 and 100 mg L^{-1} (optimal range established by FAES). Therefore, the sodium concentration in all evaluated samples was trace (0.47 ± 0.03 to 10.68 ± 0.64 mg g^{-1}) (see Table 1). It is observed that all RSD values of repeatability and intermediate precision were less than 16.6%, indicating that the analytical procedure obtained adequate precision in accordance with the Horwitz Trumpet.

Table 1 presents the sodium concentrations of the protein supplements (in mg of sodium per g of supplement).

From Table 1, it can be observed that the method achieved good repeatability, as the analyte sodium showed satisfactory RSD values (< 16.5%), indicating low dispersion among the results. As for intermediate precision (which evaluates variations within the laboratory due to changes in analysts and analysis days), the RSD values were below 16.6%, indicating good agreement between independent results. Intermediate precision is recognized as the most representative of result variability within a single laboratory. Therefore, the proposed analytical procedure is considered to have good precision, indicating reliability in the analysis of sodium in protein supplements.

Table 1. Precision (repeatability and intermediate precision) of the results obtained for sodium in protein supplements (mean ±SD, n = 3).

Samples	Average Na concentration (mg g^{-1})	Repeatability RSD (%)	Average Na concentration (mg g^{-1})	Intermediate precision RSD (%)
S1	1.70 ± 0.10	5,8	–	–
S2	1.62 ± 0.27	16.5	–	–
S3	2.11 ± 0.29	13.8	2.61 ± 0.08	14.1
S4	3.84 ± 0.49	12.6	5.04 ± 0.18	16.6
S5	3.45 ± 0.27	7.9	3.92 ± 0.14	8.8
S6	4.08 ± 0.45	11.1	–	–
S7	0.47 ± 0.03	6.0	0.56 ± 0.03	11.7
S8	5.81 ± 0.35	6.0	–	–
S9	10.68 ± 0.64	6.0	–	–

Source: Prepared by the authors, 2025.

Finally, the sodium concentrations of the four samples analyzed for intermediate precision (S3, S4, S5, and S7) were higher than those determined during repeatability testing. Since different analysts were involved, systematic errors – such as parallax during dilution – may have slightly affected sodium estimates [13]. Additionally, it is important to note that the protein supplement samples had different flavors, and although they were properly homogenized, the matrix remained uneven.

3.2 Accuracy

Accuracy is an analytical validation parameter that measures the closeness of a method's results to a reference value. Anvisa [5] does not provide fixed acceptance ranges for analyte recovery percentages. Therefore, the intrinsic variability of the method, the working concentration, and the analyte concentration in the sample are the most important criteria for accepting recovery percentages [5]. According to Souza et al. [17], an analytical method is considered accurate if the recovery percentages fall within the range of 80% to 120%. However, this range varies across studies in the literature and may extend to 50%–120% for analytes at trace and ultra-trace concentrations.

Table 2 presents the recovery rates for sample S2 (randomly selected) by standard type. For the average of the S2 sample duplicate (after the addition of the monoelemental Na standard solution), the recovery percentage was 110%; for the addition of solid NaCl standard, the recovery percentage was 78%. Both standard additions yielded recoveries within the acceptable range for analytes at trace concentrations.

According to the results above, the proposed analytical procedure demonstrates adequate accuracy for the determination of sodium in protein food supplements. It is worth mentioning that accuracy can also be assessed using a certified reference material (CRM). The CRM consists of a highly pure standard of the analyte of interest, accompanied by a certificate that provides the associated uncertainty value. By comparing the analyte concentration results obtained in the CRM with its certified value (reference value in this standard), it is possible to evaluate the accuracy of the proposed procedure. Although CRM is more appropriate for assessing the accuracy of an analytical procedure, the high cost of these certified standards has become a limiting factor.

Table 2. Recovery values (%) of sample S2 from two types of standards

	Monoelemental Standard solution of Na			NaCl solid standard		
Sample	**Recovery (%)**	**Average Recovery (%)**	**RSD (%)**	**Recovery (%)**	**Average Recovery (%)**	**RSD (%)**
S2.1	110	110	12.9	70	78	14.5
S2.2	120			86		

Source: Prepared by the authors, 2025.

3.3 Label compliance

The truthfulness of nutritional information displayed on food labels is an important factor in ensuring product quality and consumer health. Therefore, the information presented must be clear and accurate, as it is used for nutritional understanding and for selecting dietary supplements. In Brazil, the agency responsible for regulating the information that must appear on labels is ANVISA [5]. According to the World Health Organization (WHO), the recommended daily intake of sodium is 2 grams. However, studies indicate that the Brazilian population consumes high levels of sodium, which has become a public health concern, as excessive intake of this element is a major risk factor for non-communicable chronic diseases (NCDs), such as hypertension, kidney disease, and cardiovascular disorders [18].

Table 3 presents the agreement between the sodium concentration determined by FAES (per the analytical procedure proposed in this study) and the values stated on the food label (as declared by the manufacturers).

Table 3. Compliance of the determined sodium (Na) mass with the expression on the food label (mean ± SD, n = 3).

Sample	**Portion mentioned on the label (g)**	**Mass (mg g^{-1}) of Na described on the label**	**Average mass of Na (mg g^{-1}) determined**	**Confidence interval (t_2, 95%)[a]**
S1	120	2.52	1.70 ± 0.10	1.45 – 1.95
S2	31	1.45	1.62 ± 0.27	0.95 – 2.29
S3	120	1.34	2.11 ± 0.29	1.39 – 2.83
S4	30	1.73	3.84 ± 0.49	2.62 – 5.06
S5	30	1.77	3.45 ± 0.27	2.78 – 4.12
S6	40	4.12	4.08 ± 0.45	2.96 – 5.20
S7	40	1.72	0.56 ± 0.03	0.49 – 0.63
S8	40	3.35	5.81 ± 0.35	4.94 – 6.68
S9	29	17.73	10.68 ± 0.64	9.09 – 12.27

Source: Prepared by the authors, 2025. [a]The sodium concentration stated on each food label was expressed in mg. For comparison purposes with the determined sodium concentration, the label value was converted (according to the whey portion indicated on the label) to mg g^{-1}. [a]The Student's t-value (2 degrees of freedom) was calculated considering a 95% confidence interval for triplicate samples (interval in mg g^{-1}).

Normality was assessed using the Shapiro-Wilk test, and the nine sodium concentration values from the supplements were confirmed to follow a normal distribution. The results indicate that only for samples S2 and S6 does the sodium concentration stated on the food label fall within the 95% confidence interval. In other words, the null hypothesis cannot be rejected. For the other samples, the label values lie outside the 95% confidence interval, indicating that the data sets (determined concentration vs. label concentration) are statistically different (i.e., the null hypothesis is rejected). It is important to emphasize that the analytical procedure proposed for sodium determination by FAES in protein supplements was evaluated for accuracy and precision and is therefore appropriate for assessing label compliance of the samples analyzed in this study.

Figure 5 shows the percentage of samples that comply with the food label. Of the 9 samples analyzed, 45% showed sodium concentrations higher than those indicated on the label, increasing the likelihood of exceeding the recommended daily sodium intake and the risk of non-communicable chronic diseases.

If sodium levels exceeded acceptable tolerance limits, the product could be considered non-compliant with food regulations, requiring corrective action or batch rejection. Finally, based on the results obtained, some teaching/learning actions were discussed (section 3.4) that can provide significant learning of the analytical validation concepts taught in the chemistry technical course.

3.4 Educational application

Many criticisms of traditional teaching focus on the passive role of the student, who is often treated as a mere listener to the teacher's information. Such information is almost always unrelated to the prior knowledge students have built over their lifetimes. Learning is not meaningful when there is no relationship between what the student already knows and what they are learning [19]. In this context, passive learning resulting from traditional teaching leads to disinterest among students, leaving them unable to participate in their learning and leading to a focus on memorization and observation. In this way, active methodologies oppose the traditional teaching and ensure meaningful learning [20]. The methodological procedure proposed in this work aligns with an active methodology, as it uses experimentation as an educational proposal, with problematizing and questioning at the center of the teaching-learning process. It should be noted that experimentation without these actions only perpetuates traditional teaching based on memorization and observation of phenomena [21].

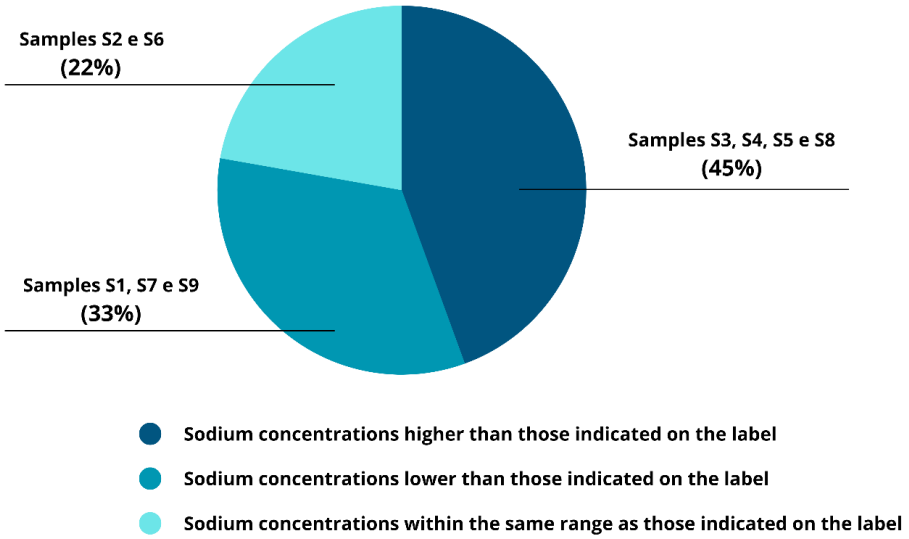

Fig. 5. Percentage of samples in relation to compliance with the food label. Source: Prepared by the authors, 2025.

In this sense, students must connect their prior knowledge to the content of the experimental activity to feel motivated to seek solutions to the questions raised [22]. Therefore, interdisciplinarity in food and nutrition education was used for this purpose, as this approach is established in the National Common Curricular Base (BNCC) [23] precisely because it overcomes the fragmentation of content and curricula, allowing the integration of content that is not generally discussed in some disciplines [24-25]. Therefore, the following didactic sequences are suggested for the application of the experimental methodology developed:

Lesson 1 (50 min): Introductory lesson on the content of validation of analytical methods. At the end of this class, the following question should be asked: "In the work of a chemistry technician, where are the contents of validation of analytical methods applied? How important is this application?" Students should be asked to write a short text on the subject for the next class. Points can be awarded to value this activity.

Class 2 (50 min): The teacher should start by leading a debate on the students' research. The debate should be used as a hook to introduce the issue of the veracity of the information on food labels (at this point, it is advisable to discuss how important this is for the diet/health of consumers, whether students should pay attention to this information when they go to the supermarket; what are the consequences and harms of this information being incorrect; which Brazilian agency carries out this control; ending with the work of the chemistry technician in this area and the relevance/application of the validation of analytical methods).

The teacher should then leave another question for the students to reflect on for the next class: "Regarding protein food supplements, a product that has been widely consumed by young people like you, is the information described on the label reliable?" Finally, the teacher should inform the students that in the next class, they will have an experimental practice to answer this question by applying the content on validation of analytical methods studied (precision and accuracy). This practical application of their knowledge will not only deepen their understanding but also motivate them to learn more. It is also recommended that students provide a list of exercises to help them recall and practice the knowledge learned in Class 1 at home. The list can also be scored.

Class 3 (1h40 min): In the experimental practice (following the methodology proposed in this work), the teacher should always revisit class discussions and allow students to participate in the entire experiment, serving as a mediator. The class should be divided into groups, and each group must analyze samples of different food supplements. This will be important for Class 4.

Once the experiment is finished, the teacher should ask for a practice report. Besides being a prerequisite for the chemistry technician, this activity is a great way to review the content, formulate arguments, and exercise writing and reflection. In this report, students need to discuss the issues covered in class, relating the validation of analytical methods to the work of the chemistry technician, especially in the context of food label quality control. The interpretation and treatment of the data obtained in the experiment is also relevant. Beyond the laboratory report, the assessment may incorporate structured rubrics and peer evaluation, providing a broader and more detailed measure of student learning.

Class 4 (50 min): As we conclude the teaching proposal, it's essential that students actively participate in the experimental practice. They will present the results they've obtained, discussing whether the samples analyzed demonstrated adequate precision and accuracy, and examining the conformity of the food label. The groups will then compare the results and discuss the importance of validating methods and the veracity of food labels.

In short, this teaching sequence must seek the student's problematizing, reflective, and investigative action in their learning process through experimentation. This will produce significant learning and awaken the student's interest in analytical chemistry.

Empirical evidence from the classroom implementation demonstrated clear improvements in students' understanding of analytical validation concepts. During the experimental activities and in their final laboratory reports, most groups correctly interpreted precision and accuracy, justified the recovery values obtained, and evaluated label compliance using the t-test. Students also showed increased engagement throughout the practical sessions, frequently connecting the analytical results to topics previously discussed in class, such as excessive sodium intake and the importance of reliable nutritional information. These findings indicate that the didactic sequence not only facilitated conceptual understanding but also strengthened students' ability to apply

analytical reasoning to real-world contexts.

4. Conclusions

The analytical procedure achieved adequate precision (repeatability and intermediate precision, with RSDs < 16.6%) and accuracy (recoveries between 78 - 110%) for the determination of sodium after acid decomposition of protein supplements. It was possible to assess that 45% of the samples presented sodium concentrations above those described on the labels, demonstrating that ingesting these supplements without medical/nutritional guidance can trigger chronic non-communicable diseases, such as arterial hypertension. In addition, the student's t-test indicated that 78% of the samples evaluated were outside the 95% confidence interval, suggesting non-compliance with these protein supplements. It is important to acknowledge that the study did not include interlaboratory reproducibility assessments, and future research is recommended to expand the analytical approach to additional nutrients or broader supplement categories. Finally, the didactic sequence proposed for teaching validation of analytical methods can be a good strategy for achieving significant learning, as it connects the students' prior knowledge with the contents of analytical chemistry. It is worth noting that the lack of teaching materials for analytical chemistry has led to a search for more appropriate educational materials for the technical chemistry course, enabling students to better visualize the concepts covered.

Author Contributions

Camila de Paula Bandoli Gomes contributed to conceptualization, methodology, investigation, data curation, and writing – original draft. Mylena de Souza Figueiredo contributed to methodology, investigation, formal analysis, visualization, and writing – review & editing. Isabelle Lucas Braga Perin contributed to resources, validation, visualization, and writing – review & editing. Patricia Gon Corradini contributed to supervision, project administration, resources, and writing – review & editing. Mario Ferreira Conceição Santos contributed to supervision, conceptualization, funding acquisition, and writing – review & editing. Murilo de Oliveira Souza contributed to conceptualization, methodology, formal analysis, supervision, and writing – review & editing.

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