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Laboratory Experiment

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Determination of Calcium in Dietary Supplements: Statistical Comparison of Methods in the Analytical Laboratory

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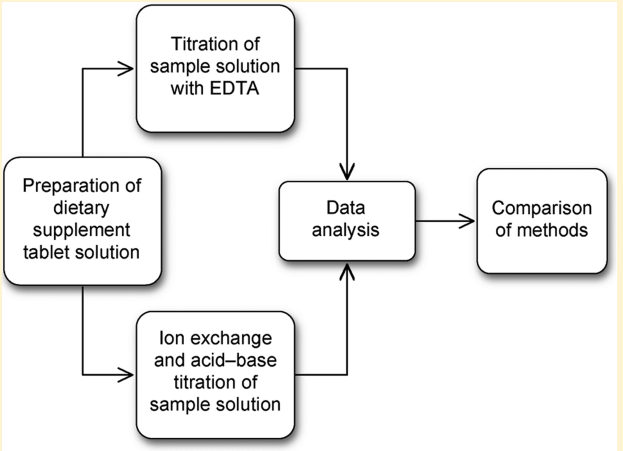
Supporting Information

ABSTRACT: A laboratory experiment is described in which students compare two methods for the determination of the calcium content of commercial dietary supplement tablets. In a two-week sequence, the sample tablets are first analyzed via complexometric titration with ethylenediaminetetraacetic acid and then, following ion exchange of the calcium ion present for hydronium ion, by acid–base titration with sodium hydroxide. Upon completion of the laboratory work, students pool their data with classmates and perform a statistical analysis to determine whether the average values of the calcium content obtained by the two methods are equivalent. When taken together with such considerations as analysis time, accuracy, simplicity and ease of use, susceptibility to interference, and waste generation, these results enable the students to evaluate the relative merit of the two approaches to calcium determination. In so doing, they are introduced to an important aspect of "real-world" chemical analysis, namely, selection of the method best suited for the determination of a particular analyte in a given sample.



KEYWORDS: *Second-Year Undergraduate, Analytical Chemistry, Hands-On Learning/Manipulatives, Acids/Bases, Ion Exchange, Titration/Volumetric Analysis*

INTRODUCTION

An important but often inadequately emphasized part of undergraduate analytical chemistry laboratory courses is the assessment of the relative merit of different analytical methods. Too often, in fact, students are simply presented with a sample and a detailed description of a method for its analysis, leaving little opportunity for critical thinking.

Despite its obvious importance, textbooks only infrequently emphasize method comparison,[1,2] and published laboratory experiments on this topic are not especially numerous. In 1966, Hanrahan[3] described an experiment in which unknown samples were analyzed for xylenes by infrared and UV–visible spectroscopy, along with gas chromatography. A subsequent paper by Steinfeld[4] outlined a project involving a comparison of the accuracies of gravimetric, volumetric, and electrochemical methods for the determination of silver ion. At around the same time, Beilby[5] detailed an experiment in which potentiometric and photometric approaches to the detection of an end point in a coulometric titration were compared. Later work by the same author[6] employed copper-containing alloys as the basis of an exercise in which a number of methods commonly included in analytical laboratory courses, among them polarography, atomic absorption spectroscopy, potentiometry, and iodometric titration, were compared. More recently, Harrison and Peterman[7] outlined a series of experiments involving the comparison of either two different instrumental methods (e.g., enzyme analysis by NMR and UV–visible spectroscopy) or a "wet" method to an instrumental approach to the same analysis (e.g., determination of iron in an ore by potentiometric titration and atomic absorption spectroscopy). Similarly, Edmiston[8] described an experiment for the advanced analytical laboratory involving the comparison of the performance characteristics of various instruments when applied to the determination of a given analyte. Finally, in 2011, Revell[9] described an experiment in which two methods for the separation of the components of commercial analgesic tablets, two-base extraction and column chromatography, were critically evaluated. In all of these instances, the pedagogical advantage of performing a method comparison over carrying out the same analyses in isolation was noted.

With this in mind, we have developed and implemented a two-week laboratory experiment in which the calcium content of commercial dietary supplement tablets is determined by two different approaches. In the first week, the analysis is accomplished by complexometric titration with ethylenediaminetetraacetic acid (EDTA). In the second, passage of the sample through a column of a strong cation-exchange resin to exchange the calcium ion present for hydronium ion is followed

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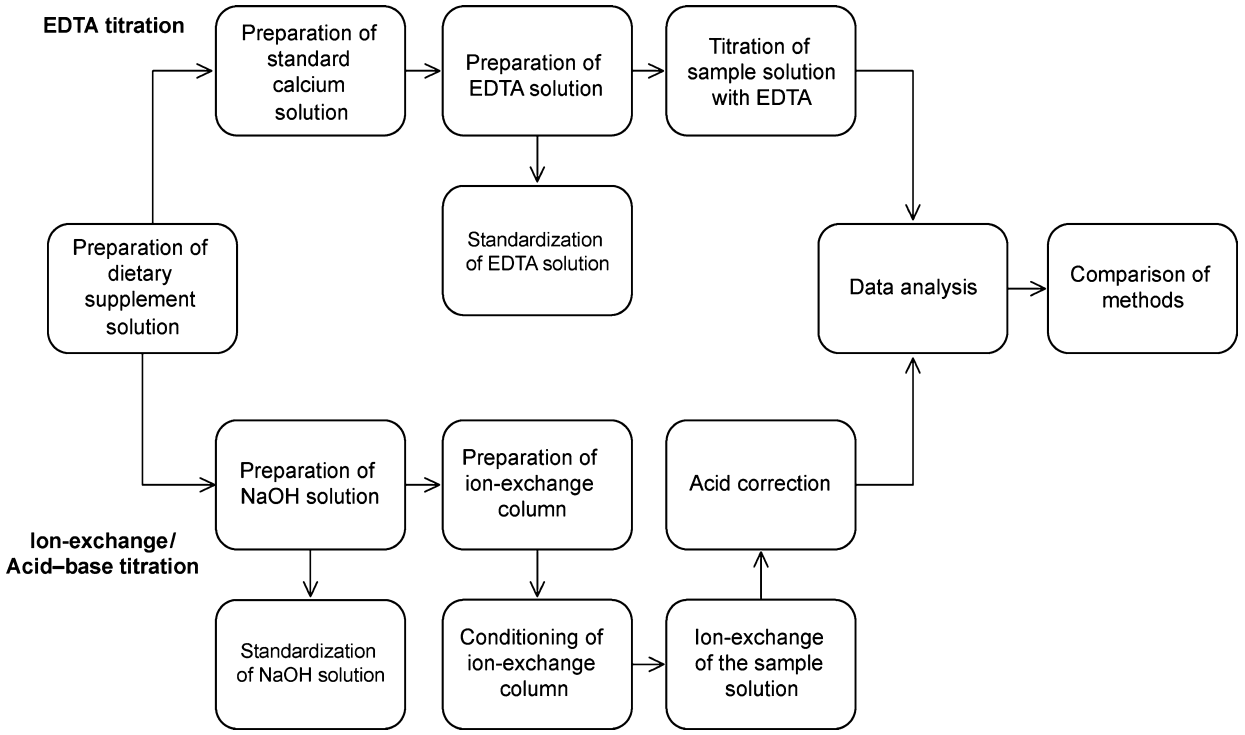

Figure 1. Overview of the procedures for determining the calcium content of dietary supplement tablets.

by acid–base titration with standard sodium hydroxide. The students then evaluate the efficacy of the two approaches in terms of the accuracy of the results (i.e., the deviation vs the label values) and such considerations as analysis time, ease, and waste generation. Lastly, after pooling their data with classmates, the students perform a statistical analysis to determine if the results obtained by the two methods are equivalent. This sequence has been tested and modified over the course of four semesters and has been successfully performed by more than 300 students to date.

EXPERIMENTAL DETAILS

Materials and Methods

Figure 1 summarizes the procedures employed for the analysis of the dietary supplement tablets. A detailed discussion of them, along with a description of the materials required, is provided in the Supporting Information section. It is important to point out that the time required for completion of the entire experiment is approximately 6 h for a typical student. When two 4 h laboratory periods are available (as is the case for our students), this clearly poses no problem. To complete the work in a pair of 3 h laboratory periods, however, students must begin the ion exchange/acid–base titration experiment during the first week of the sequence. For example, the students could prepare, condition, and wash the ion-exchange column. In the alternative, the sodium hydroxide solution could be prepared and standardized. In the former case, the column must be carefully sealed to prevent it from becoming dry during storage, while in the latter case the solution must be protected from atmospheric carbon dioxide. If significant progress can be made on either of these tasks during the first week, then no difficulty will be encountered in completing the remainder of the ion-exchange (IX) acid–base titration procedure in the second.

Data Analysis

Following completion of the laboratory work, all student results for a given brand and method are pooled, and a Grubbs' test is applied to the data to determine if any values can be considered statistical outliers. Those so identified are removed from the data set for all subsequent calculations. After outlier removal, the mean, standard deviation, relative standard deviation, confidence interval at the 95% level, and the percent deviation vs the label value are calculated for each brand of tablet for each method. Next, an F test is performed to determine if the standard deviations associated with the two procedures are statistically different for a given brand. Finally, a Student's *t* test is applied to determine if the two methods yield statistically equivalent results for the calcium content of a given brand.

HAZARDS

This experiment involves strong acids and bases, which are corrosive. The hazards of the prepared solutions are minimal at the low concentrations used, however. Nonetheless, goggles should be worn at all times during this experiment. In addition, the Material Safety Data Sheet (MSDS) for each reagent should be consulted prior to the start of any work.

RESULTS AND DISCUSSION

Table 1 summarizes the results obtained by the students who performed the experiment during the Spring 2013 semester. As in prior semesters, the precision and accuracy of the ion-exchange/acid–base titration approach were found to be poorer than for the complexometric titration, not an unexpected result given the greater number of steps (and thus the greater opportunity for error) involved in the former method. Despite this, the results were not found to be statistically different at the 95% level of confidence.

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Table 1. Representative Student Results for Determination of the Calcium Content of Two Brands of Dietary Supplement Tablets by Complexometric Titration, and Ion Exchange and Acid−Base Titration

	Citracal (Calcium Citrate with Vitamin D)[a]		Store Brand[b]	
	Label Value: 315 mg Ca^{2+}/Tablet		Label Value: 250 mg Ca^{2+}/Tablet	
Sample	EDTA[c]	IX[d]	EDTA[c]	IX[d]
Average (mg Ca^{2+}/tablet)	326	287	266	264
Standard Deviation	27	85	24	90
%RSD	8.3	30	9.0	34
Confidence Interval	326 ± 13	287 ± 44	266 ± 13	264 ± 45
Deviation, %[e]	+3.49	−8.89	+6.40	+5.60
$t_{calculated}$; $t_{tabulated}$[f]	1.81; 2.095		0.091; 2.086	
Statistical Difference?[g]	No		No	

[a]"Brand A". [b]"Brand B". [c]Results for complexometric titration, Spring 2013 Semester. [d]Results for ion exchange and acid−base titration, Spring 2013 Semester. [e]The expected value used in the calculation of % deviation was that found on the label for each brand. [f]The calculated *t*-value was compared to the literature value[10] at the 95% confidence level for 19 degrees of freedom for brand A and 20 degrees of freedom for brand B. [g]If $t_{tabulated} > t_{calculated}$, then there is no statistical difference between methods.

CONCLUSIONS

This experiment, in addition to illustrating many of the criteria employed by practicing analytical chemists in choosing one method for sample analysis over another, offers several additional benefits. First, it employs relatively inexpensive reagents and equipment. In addition, by making use of "real world" samples, it increases the level of student interest.[11] Finally, it illustrates the application of statistical methods in chemical analysis at a level of sophistication greater than that which characterizes many previously described statistical exercises for the undergraduate laboratory.[12−15]

While we have chosen to employ dietary supplements containing calcium citrate as the unknown for this experiment (a result of their low cost and ready availability), we see no inherent reason that the procedures outlined will not work equally well for those containing the corresponding lactate or carbonate. In fact, prior work by one of the authors[16] has demonstrated that the ion-exchange/acid−base titration approach provides satisfactory results for supplements based on calcium lactate.

ASSOCIATED CONTENT

Supporting Information

A detailed description of the experimental procedures, a set of sample calculations, an example student handout, and a description of the statistical treatment of the data obtained are provided. This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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