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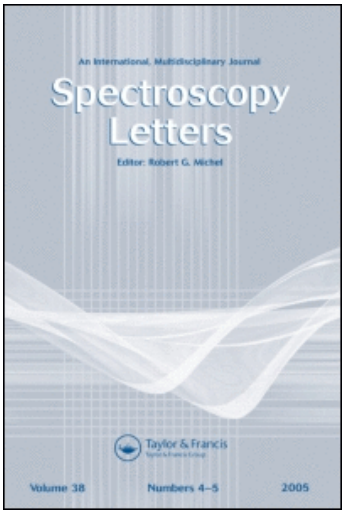

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Undergraduate Analytical Chemistry: Method Development and Results of the Analysis of Bismuth in Pharmaceuticals

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ABSTRACT Three different methods were developed and employed to analyze the concentration of bismuth subsalicylate in an oral liquid pharmaceutical suspension. These methods were UV-Visible spectroscopy utilizing the external calibration method and the standard addition method, flame atomic absorption spectroscopy, and complexometric titration with EDTA. All three methods were conducted within one 3-hr period by undergraduate students in analytical chemistry laboratory class settings and produced results within the allowable range of 90.0–110.0% as listed in the United States Pharmacopeia. Differences in sensitivity, precision, sample size, time, and reagent use between the three methods were observed.

KEYWORDS bismuth, bismuth subsalicylate, EDTA titration, flame atomic absorption spectroscopy, method development, United States Pharmacopeia, UV-Vis spectroscopy

INTRODUCTION

Pharmaceutical formulations of bismuth compounds have been used for generations to soothe an upset stomach.[1] The conditions treated also include gastrointestinal disorders, gastric or duodenal ulcers, diarrhea, and syphilis.[2] A number of different bismuth salts have been used as the active ingredient in the formulations. These salts include the subnitrate, subcarbonate, subgallate, subsalicylate, tartrate, and subcitrate salts. The term "sub" refers to the high oxygen content and the existence of "Bi-O moieties."[2] Once ingested, bismuth can be found in the blood, urine, and feces. Among the organs, the kidneys have the highest concentration of bismuth. A variety of bismuth compounds have been linked to toxic effects in humans.[3]

The quantitative analysis of bismuth in tablet, powder, or cream formulations and oral liquid pharmaceutical suspensions involves titrimetric or spectroscopic methods that are well documented.[4–13] The concentration of bismuth in a solution can be determined through a complexometric titration with ethylenediaminetetraacetic acid (EDTA).[10,11,14] Many indicators have been successfully used[11] for this titration, with xylenol orange being the preferred choice of the United States Pharmacopeia (USP) and the British

At the time of research, coauthors Brent Ferguson and Rebekah Kimbrough were undergraduate students.

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Pharmacopoeia.[10,14] Spectroscopic analyses have been done by resonance light scattering,[4] first derivative UV-Visible spectrophotometry,[5] UV-Visible spectroscopy by the analysis of the tetraiodobismuthate(III) anion,[8,10,12,13] flow injection systems,[6,7] hydride generation atomic absorption spectroscopy,[9] and flame atomic absorption spectroscopy (FAAS).[10]

The only article describing the analysis of bismuth in pharmaceutical tablets in the analytical chemistry teaching laboratory[15] determines the concentration of bismuth through anodic stripping voltammetry and not titrimetric or spectroscopic methods. There are no articles in the chemical education literature describing the analysis of the oral suspensions. The liquid suspension is an excellent sample for analysis by students since it can be purchased at any drug or convenience store. The salt commonly used in the oral liquid pharmaceutical suspension is the bismuth subsalicylate, $C_7H_5BiO_4$ (BSS), which is a white, odorless powder that is insoluble in water.[16] The concentration listed on most, if not all, of the commercial products[17] is 262-mg BSS per 15 mL or 262-mg BSS per caplet or tablet for the regular strength. It is noteworthy that although bismuth is a heavy metal and a well-used pharmaceutical agent, there is still much that is not understood about the mechanism of action.[18]

This paper describes the experimental development phase performed by undergraduate research students and a faculty member for the analysis of bismuth in liquid suspensions. The subsequent results were determined primarily by students in the analytical chemistry laboratory courses by both titrimetric and spectroscopic methods principally following the methods provided by the USP.[10] The analyses performed include both the standard addition method and the external calibration method. An internal standard method was not performed, but one can be found in the literature.[19] All three experiments described can be accomplished in one 3-hr laboratory period and provide the students with the experience of examining the sensitivity, precision, sample size, time, and reagent use of three independent analytical methods (UV-Vis spectroscopy, FAAS, and EDTA titration) as typically employed for certified reference materials.

MATERIALS AND METHODS

All reagents were analytical grade and used as purchased. These reagents include concentrated nitric acid (Fisher, Fair Lawn, NJ), 1000-ppm Bismuth (Bi) Standard (Spectro Pure, Arlington, TX), and those listed below. The following solutions were prepared: 10% ascorbic acid (Fisher, Fair Lawn, NJ), 20% potassium iodide (Fisher, Fair Lawn, NJ), 1-M nitric acid, 0.05-M EDTA (Acros Organics, Geel, Belgium), 0.1% xylenol orange (Sigma–Aldrich, St. Louis, MO), and 0.01-M nitric acid. All solutions were stored at room temperature.

Density/Specific Gravity

A Mettler Toledo Densitometer 30PX (Columbia, OH) instrument was used to measure density or specific gravity. Five density readings of the bismuth oral pharmaceutical suspension were taken and averaged. Alternatively, a density of 1.02 g/mL could be used.

Sample Solution Preparation

A weight of approximately 12.5 g (recorded to four decimal places) of the bismuth oral suspension was transferred quantitatively into a 250-mL volumetric flask and diluted with 1-M nitric acid. This sample was further diluted by pipetting 10.00 mL into two 100-mL volumetric flasks. Into one, 1.000 mL of the 1000-ppm Bi standard solution was added (spiked). Both sample solutions were diluted with 1-M nitric acid to the line.

Flame Atomic Absorption Spectroscopy

Using the above un-spiked sample solution, 500 μL was diluted in a 10-mL volumetric flask with 0.01-M HNO_3. Standards of 0-, 2.5-, and 5-ppm Bi were prepared from the 1000-ppm Bi standard and diluted with 0.01-M HNO_3. A Varian SpectrAA 220 Fast Sequential Flame Atomic Absorption Spectrometer (FAAS, Palo Alto, CA) was used for analysis of bismuth. The Bi wavelength used was 223.1 nm with a slit-width of 0.2 nm. The hollow cathode lamp current was 10.0 mA, and the background corrector was on. The Air-Acetylene flame (10-cm head) had flow rates of 13.50 L/min of air and 2.0 L/min of acetylene. Only one result per standard and sample was obtained due to using the PROMT mode of data collection, where the average result is reported only when the precision for both standards and samples is less than 1%. The use of PROMT greatly speeds up the analysis.

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UV-Vis Spectroscopy

Using both of the above spiked and un-spiked sample solutions, 500 μL of each was added to two 10-mL volumetric flasks, respectively. A standard at 2.5-ppm Bi was prepared by adding 25 μL of the 1000-ppm Bi into a third 10-mL volumetric flask. To an empty 10-mL volumetric flask and the three described above, 2.000 mL of 10% ascorbic acid solution and 5.000 mL of 20% potassium iodide solution were added. Deionized water was used to dilute to the line. A Varian Cary 4000 (Palo Alto, CA) was used for development work by the undergraduate research students, and a Varian Cary 50 (Palo Alto, CA) with fiber optic dip probe was used for the routine analyses by the students in the analytical laboratory courses. The tetraiodobismuthate(III) anion, which imparts a yellow color to the solution, was measured at 464 nm by the Cary instruments.

EDTA Titrations

About 10–13 g (recorded to four decimal places) of the bismuth oral suspension was added to a weighed Erlenmeyer flask weighing less than 85 g. This was repeated two more times. In a fume hood, each of the three flasks received 2.5 mL of concentrated nitric acid. By swirling, all of the pink color disappeared, and 40 mL of deionized water was subsequently added. To each, using a plastic transfer pipette, we added one mL of xylenol orange indicator, which turned the solutions red. The solutions were titrated with EDTA of known concentration to a yellow endpoint. One more mL of xylenol orange indicator was added at the end to ensure that the end point had been reached.

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RESULTS AND DISCUSSION

Method Development

The procedure in the US Pharmacopeia[10] requires that (1) 10 g of the oral suspension be weighed out into a 200-mL volumetric flask and dissolved and diluted with 1-M HNO_3. Then, (2) 10 mL of that solution should be further diluted to 100 mL with 1-M HNO_3. Subsequently, (3) a volume containing 0.9 mg of BSS is to be placed in a 50-mL volumetric flask. Rather than using the 200-mL volumetric flask as described in the US Pharmacopeia[10], a 250-mL volumetric flask was used. In addition, rather than use a 50-mL volumetric flask, 10-mL volumetric flasks were used. Since the suspension is listed as 262-mg BSS per 15 mL rather than per a nominal mass, the density of the solution is required in order to determine what volume to measure in Step 3.

Three methods to determine the density were employed during method development by the undergraduate research students and the faculty member. Method 1 entailed using a tared 10-mL graduated cylinder, adding 2 mL of the liquid, and recording the mass. The graduated cylinder was tared, another 2 mL was added, and this was repeated until five measurements were made. Method 2 employed a micropipette with a tared weigh-boat and pipette tip (both tared together). Either 0.5 mL or 1.0 mL was pipetted into the pipette tip, and this was manually removed from the micropipette and placed on the tared weigh-boat to determine the mass. Five trials were performed. Method 3 was the procedure listed in the US Pharmacopeia and used the densitometer.[10] A Mettler Toledo Densitometer 30PX is an oscillating transducer density meter and uses a magneto- or piezo-electric excitation system to cause the U-shaped tube holding the sample to oscillate at a frequency characteristic of the sample.[10]

The density of the oral suspension formulation varies with the brand and strength. For one particular brand, Method 1 found a density of 1.01 ± 0.05 g/mL, and Method 2 found densities of 1.022 ± 0.005 g/mL and 1.04 ± 0.02 g/mL for 0.5- and 1-mL samples, respectively. Using Method 3, we found the density to be 1.016 ± 0.002 g/mL for the same brand. Clearly, Method 3 has the best precision, and the use of this instrument should be encouraged, although it is not necessary for the implementation of this laboratory experiment. The results obtained by students in the teaching laboratory, using Method 2, were very poor due to their inexperience with the operation of the micropipette. The students would not allow for the high viscosity of the solution and thus would not slowly release the plunger allowing for the accurate suction by the micropipette. Thus, if density is not to be measured, a value of 1.02 g/mL can be used, but it is highly suggested to use Method 3 for each sample investigated.

The USP[10] method requires centrifugation of the sample solution. Methods of centrifugation, filtration through 0.45-micron filters, an extended digestion time (overnight), and the standard addition method were investigated by the undergraduate research students and were found to be unnecessary due to the

resulting high accuracy and precision of all three methods. One standard addition method remains to illustrate the method to the undergraduate students in the course. The amount of undergraduate research time and faculty time devoted to this development was approximately 6 hr/week for three semesters. The two classes that have experienced this experiment as a laboratory procedure comprised 10 students in a new course entitled Pharmaceutical Analysis in fall 2008 and 12 students in Quantitative Analytical Chemistry Laboratory in summer 2009.

Flame Atomic Absorption Spectroscopy

The undergraduate research students developed external calibration curves to determine the linearity of the methods using the 1000-ppm Bi standard and subsequent dilutions. Calibration solutions were made in the ranges 0–50-ppm Bi (0-, 5-, 10-, 20-, 50-ppm Bi) and 0–10-ppm Bi (0-, 1-, 2-, 4-, 5-, 6-, 8-, 10-ppm Bi). Linearity was only observed between 0 and 20 ppm. The linearity and R^2 improved from 0 to 20 ppm, from 0 to 10 ppm, and from 0 to 5 ppm. All subsequent analyses were performed between 0 and 5 ppm, using only 0-, 2.5-, and 5-ppm standards.

The results for the external calibration method ($R^2 = 0.9994$) and calculated value are found in Table 1. This result (260-mg BSS/15 mL) is 99.2% of the labeled value of 262-mg BSS per 15 mL. Precision is assumed to be 1% or less due to the use of the PROMT function on the FAAS.

UV-Vis Spectroscopy

The USP[10] instructions required the creation of a 10.00-ppm Bi standard solution. The analyses proceed by following the ratio method. The undergraduate research students showed that linearity was observed up to 10 ppm with an R^2 value of 0.9999. Thus, further analyses by students were conducted using a blank and a 2.5-ppm Bi standard. This is similar to the USP method whereby only one standard is used. Table 1 lists the slope and calculated values of the analysis by both the external calibration method and the standard addition method.[20] These values of $102 \pm 1\%$ and $97 \pm 2\%$ are within the allowed labeled limits of 90.0% and 110.0%.[10] Note that the standard deviations are very small.

EDTA Titrations

The average with standard deviation is 256 ± 9-mg BSS/15 mL, which represents $98 \pm 3\%$ of the listed amount. The EDTA titration method had the largest standard deviation of all three methods as seen in Table 1.

Learning Outcomes

Specific learning outcomes derived from this experiment include the following:

1. Explain how three different independent analytical methods can be used to determine the same answer as typically employed in certified reference materials.
2. Describe the relative differences in sensitivity, precision, sample size, time, and reagent use between (a) the wet-chemical method of EDTA titrations and (b) UV-Vis spectroscopy and FAAS.

All students in the two classes were able to have one of the three methods provide them with the expected values. In their discussions, the students were able to describe why the other methods did not provide the expected values, and, in most cases, the cause was the skill level of the student with the use of the micropipettes, etc.

TABLE 1 **Results by Three Analytical Methods for Bismuth Subsalicylate, BSS, in an Oral Liquid Pharmaceutical Suspension**

Parameters	External calibration FAAS	External calibration UV-Vis	Standard addition method UV-Vis	EDTA titrations
Slope (absorbance units/ppm)	0.02144	0.05697	0.06100	N/A
Concentration measured (ppm)	2.447	2.53 ± 0.02	2.39 ± 0.06	N/A
Concentration in original solution (mg BSS/15 mL)	260.	268 ± 2	254 ± 6	256 ± 9
% of stated quantity	99.2%	$102 \pm 1\%$	$97 \pm 2\%$	$98 \pm 3\%$

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In summary, the results of the students varied with their skill level. The laboratory courses are intended to increase their skill level and their breadth and depth of experiences, not to evaluate them on each laboratory exercise. Thus, while it is nice to obtain values consistent with the labeled products, not obtaining those values provides an indicator to both the student and the instructor that their skill and technique need improvement. However, it is necessary to know whether the methods, when properly executed, obtain the stated values. These results indicate that all three methods are capable of obtaining the correct values.

CONCLUSION

These experimental procedures can all be accomplished in just one 3-hr laboratory setting, providing students with the ability to see how different techniques can be used for the same purpose. The real-world sample is analyzed for both density and bismuth content. The students should be able to clearly see the difference between the EDTA titration and the spectroscopy techniques in terms of time for the analysis, sample size involved, precision, sensitivity, and amount of reagents used.

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