# Quantification of Bismuth Subsalicylate in Pharmaceutical Oral Suspensions Using Spectrophotometry and External Standard Calibration

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Date Performed: 16 February 2017
Date Submitted: 20 April 2017

**Introduction:**

Bismuth-containing pharmaceuticals are commonly used to treat symptoms such as nausea, heartburn, ulcers, and other gastrointestinal disorders. A popular brand of this type of medicine is Pepto-Bismol, an antacid whose active ingredient is bismuth subsalicylate (BSS). Research to determine the exact mechanism for how BSS works is still being done, but many researchers seem to think that it reduces inflammation in the stomach, and coats the stomach and intestines in order to protect them from stomach acid. One of the known benefits of BSS is that it is effective in vivo over a wide pH range. In the stomach, a low pH environment, the dissociation bismuth from salicylate results in bactericidal activity[4] which helps kill certain bacteria, such as *E. coli.*

Another benefit of BSS in digestive aids is that it may be administered orally rather than intravenously, making it easy for on-the-go or at-home treatment. Bismuth subsalicylate in Pepto-Bismol is present at a concentration of 525mg/30mL. The dosage for ages 12 and up is 30mL every hour without exceeding 8 doses within 24 hours. However, the concentration of BSS is potentially damaging to more sensitive gastrointestinal tracts, such as those found in a dog or young child. Therefore, dosage for children under the age of 12 and for dogs is less than the normal directed use to decrease the amount of BSS within the digestive tract. For example, it is recommended that a dog only receive 1 teaspoon, or about 5mL, of Pepto-Bismol for every 10 pounds once every 6-8 hours.[2]

Dosage for each pharmaceutical depends on the maximum amount of absorption of BSS within the gastrointestinal tract as well as the maximum amount of BSS able to be present in the system without being harmful. It is therefore important for researchers to be able to quantify the amount of bismuth subsalicylate in each digestive aid.

The external standard technique allows the quantification of an unknown analyte concentration in a sample by creating several solutions with varying amounts of known analyte concentrations and measuring the absorbance in a spectrometer. According to Beer's Law, absorbance $A$ (unitless) is related to the molar concentration $c$ (with units M) of a sample by the formula:

$$A = b\varepsilon c \tag{1}$$

Where $b$ is the sample pathlength (cm), and $\varepsilon$ is the molar absorptivity ($M^{-1}cm^{-1}$). We can therefore use the slope of a calibration curve plotting absorbance versus analyte concentration to determine an unknown concentration in a sample.

In the case of bismuth subsalicylate, no chromophore is available for absorbance measurements in spectrophotometric analysis. In order to quantify the amount of BSS present in a sample of Pepto-Bismol, the bismuth subsalicylate was "digested" by the acid $HNO_3$ causing dissociation and freeing bismuth (Bi) in solution. Adding potassium iodide (KI) to the solution allows Bi to react with iodine and form tetraiodobismuthate ($BiI_4^-$)– an anion that has a measurable absorbance in the visible region

($\lambda_{max} \sim 464nm$). Using the absorbance data from $BiI_4^-$ samples, we can quantify the amount of Bi in the digested sample as well as the concentration of bismuth subsalicylate in Pepto-Bismol. The purpose of this experiment was to demonstrate the accuracy of the external standard method in determining unknown analyte concentration by comparing the experimental concentration of BSS in Pepto-Bismol to the reported value on the bottle, 525mg/30mL.

**Experimental Procedure:**

*Solution and Sample Preparation*

Following the protocol outlined in Koether's procedure[3], four 25mL external standard solutions were prepared by diluting 57.42 $\mu M$ Bi with 2mL of 10% ascorbic acid, 5mL of 20% KI, and 1M $HNO_3$ diluent. The solutions were prepared in a 25mL volumetric flask using micro- and volumetric pipettes to transfer each reactant. The volume of Bi stock measured in the four samples was 10mL, 6mL, 4mL, and 2mL (Table 1). A fifth solution of $HNO_3$, KI, and ascorbic acid was prepared and served as the blank sample.

Table 1. Preparation of 57.42 $\mu M$ Bismuth External Standard Solutions in 1M $HNO_3$

| External Standard Solution No. | Total Volume (mL) | Volume HNO3 (mL) | Volume Bi stock (mL) | Volume ascorbic acid (mL) | Volume KI (mL) | Con. Of Ext. Std. Prepared ($\mu M$) |
|---|---|---|---|---|---|---|
| **I** | 25 | 8 | 10 | 2 | 5 | 22.968 |
| **II** | 25 | 12 | 6 | 2 | 5 | 13.7808 |
| **III** | 25 | 14 | 4 | 2 | 5 | 9.1872 |
| **IV** | 25 | 16 | 2 | 2 | 5 | 4.5936 |
| **Blank** | 25 | 18 | 0 | 2 | 5 | 0 |

*Instrumentation and Instrumental Parameters*

The absorbance was measured in a Spectronic 20 Genesys spectrometer using two clean cuvettes; one for the blank, and one containing the Bi external standard solution. The wavelength of maximum absorbance for the Bi solution was determined to be 464nm. Absorbance measurements for each of the four external standard solutions were taken at the maximum absorbance value, blanking the spectrometer after each sample measurement.

*Preparation and Analysis of Bi External Standard Curve*

Three measurements for each external standard sample were taken, and the average absorbance for each solution was recorded (Table 3). A calibration curve was prepared by plotting the average absorbance versus the known concentration of Bi (μM) in each external standard solution. A linear regression analysis was performed to determine the equation and slope of the calibration curve.

*Analysis of Bismuth Subsalicylate in Pepto-Bismol Suspension*

A digested oral suspension of Pepto-Bismol was made by diluting 12.5g of Pepto-Bismol per 250mL of solution. The unknown bismuth concentration sample was prepared by diluting 0.100 mL of the digested oral suspension to a clean 25mL volumetric flask using a p1000 micropipette. The sample was diluted in 2 mL of 10% ascorbic acid, 5 mL of 20% KI, and 17.9 mL of 1M $HNO_3$, using respective volumetric pipettes to transfer each reactant. A clean cuvette was filled 1/3 full with the unknown solution and the absorbance was recorded and averaged for three separate measurements (Table 4). The calibration curve recorded for Bi standard solutions was used to determine the concentration of tetraiodobismuthate in the unknown sample of Pepto-Bismol. The concentration of bismuth subsalicylate in the original Pepto-Bismol oral suspension was then determined using Eq. (2) and the molecular weights and density values reported in Table 2.

$$M_1V_1 = M_2V_2 \tag{2}$$

Where $M_1$ and $M_2$ are the concentrations (molarity) of the analyte before and after dilution, respectively; and $V_1$ and $V_2$ are the volumes of solution before and after dilution, respectively. The calculated concentration of bismuth subsalicylate was determined in *mg of BSS/30 mL of Pepto-Bismol* and compared to the theoretical value reported on the Pepto-Bismol label, 525mg/30mL by calculating the percent error:

$$\frac{|Theoretical - Experimental|}{Theoretical} \times 100\% \tag{3}$$

Table 2. Molecular Weights and Density used to Determine the Concentration of BSS in Pepto-Bismol

| | |
|---|---|
| **Molecular Weight $\boldsymbol{BiI_4^-}$** | 716.58 g/mol |
| **Molecular Weight $\boldsymbol{Bi}$** | 208.98 g/mol |
| **Molecular Weight $\boldsymbol{Bismuth\ Subsalicylate}$** | 362.09 g/mol |
| **Density of Pepto-Bismol** | 1.02 g/mL |

*Safety Considerations*

Nitric acid, $HNO_3$, is a corrosive acid and a strong oxidizing agent; proper precautions were taken to prevent contact of $HNO_3$ with skin and eyes. Gloves, goggles, and a lab coat were worn at all times during the experiment. Additionally, all solutions were disposed in the acidic waste container.

**Results:**

The absorbance measurements for each external solution, and the calibration curve prepared from the measurements are shown in Table 3 and Fig.1 below.

Table 3. Absorbance Values Recorded for Bi External Standard Samples.

| External Standard No. | Conc. of ext. std. (μ*M*) | A (trial 1) | A (trial 2) | A (trial 3) | Avg. A |
|---|---|---|---|---|---|
| **I** | 22.968 | 0.278 | 0.276 | 0.274 | 0.276 |
| **II** | 13.7808 | 0.169 | 0.171 | 0.173 | 0.171 |
| **III** | 9.1872 | 0.109 | 0.11 | 0.114 | 0.111 |
| **IV** | 4.5936 | 0.067 | 0.065 | 0.064 | 0.0653 |

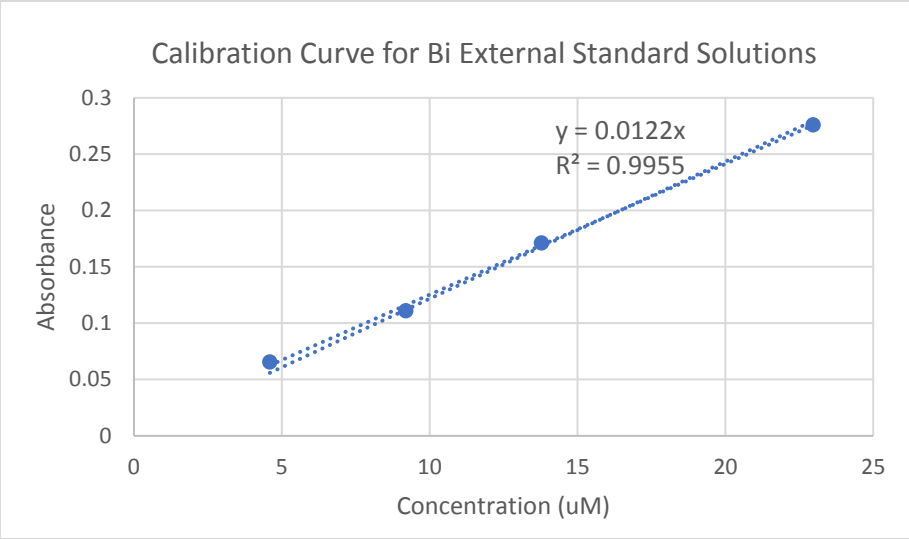


Figure 1. Calibration curve plotting absorbance of varying, known concentrations of Bi in four external standard solutions. Equation, slope, and $R^2$ values displayed on the graph.

The absorbance values of the unknown concentration of digested oral suspension are represented in Table 4, followed by sample calculations of determining the original concentration of BSS in Pepto-Bismol using the calibration curve.

Table 4. Absorbance Measurements of Unknown Bi Concentration in Digested Pepto-Bismol Suspension.

| | A (Trial 1) | A (Trial 2) | A (Trial 3) | Average A |
|---|---|---|---|---|
| Digested Bi solution | 0.136 | 0.125 | 0.128 | 0.12967 |

Table 5. Concentration of Bi in Digested Suspension and Concentration of BSS in Pepto-Bismol Compared to Reported BSS Concentration on Pepto-Bismol Label.

| | |
|---|---|
| **Conc. of Bi in original digested sample ($\mu M$)** | 10.6287 $\mu M$ |
| **Concentration of Bismuth Subsalicylate in Pepto Bismol (mg/30mL)** | 521.976mg/30 mL |
| **Reported Concentration of Bismuth Subsalicyclate in Pepto-Bismol (mg/30mL)** | 525mg/30mL |
| **% Error** | 0.576% |

*Sample Calculations*

Used to determine concentration of Bi in external standard solutions and unknown digested Pepto-Bismol suspension:

$$M_1V_1 = M_2V_2 \quad (2)$$

Used to determine concentration of tetraiodobismuthate in unknown sample (from calibration curve):

$$y = 0.0122x \quad (3)$$

To determine concentration of bismuth subsalicylate in Pepto-Bismol:

$$\left(\frac{mol}{L}Bi\right)\left(\frac{326.09\,g}{mol}Bi\right)\left(\frac{0.001L}{0.001g}\ Bismuth\ Subsalicylate\right)(30mL\ Pepto-Bismol) \quad (4)$$

**Discussion:**

The purpose of this experiment was to demonstrate the accuracy and usefulness of the external standard method in determining unknown bismuth subsalicylate concentrations in digestive aids, namely, Pepto-Bismol. Because bismuth subsalicylate does not have a suitable chromophore for spectrophotometric analysis, the Pepto-Bismol was digested in $HNO_3$ and complexed with KI to form tetraiodobismuthate. The $BiI_4^-$ anion has a maximum absorbance value at a wavelength of 464nm, which exactly matched the $\lambda_{max}$ determined in this experiment. The four external standard solutions prepared had Bi concentrations between 4.59µM and 22.97µM, with the more concentrated solutions having a higher absorbance value (Table 3). The calibration curve for the Bi external standard solutions was linear, with absorbance and Bi concentration positively correlated.

The average absorbance for the unknown sample, 0.129, fell between the absorbance measurements of the second and third external standard solutions, having Bi concentrations of 13.781µM and 9.187µM, respectively. Therefore, based on the calibration curve and the average absorbance of the unknown sample, we expected the concentration of Bi in the digested suspension to be somewhere between 9µM and 13µM. Dividing the average absorbance of the unknown sample by the slope of the line of the calibration curve (0.0122), gave a concentration of Bi in the original digested sample of 10.6287 µM. This concentration is within the predicted range of concentration determined

from the second and third external standard solutions, which supports the use of external standards and calibration curves in determining unknown concentrations.

The concentration of bismuth subsalicylate in Pepto-Bismol was determined to be 521.976mg/30 mL, which is a 0.576% error from the concentration of BSS on the bottle of the commercial product, 525mg/30mL, as can be seen in Table 5. These results support that the external calibration method is a good technique to determine unknown analyte concentrations in solutions. The accuracy of the external standard technique is important because it provides a quick, simple method of determining concentrations of active ingredients in pharmaceuticals. Physicians and researchers can use this information to recommend the best possible, non-harmful dose of a given medicine based on the amount of active ingredient in the sample. For instance, Pepto-Bismol dose recommendations for ages 12 and up is 525mg/30mL, versus a dosage of 1 teaspoon/10lbs for dogs every 6 hours. Previous research has shown that the rate of gastric emptying as well as the pH of the stomach are both responsible for determining how much nondissociated BSS enters the small intestine to react with other anions and form bismuth salts.[1] Here, the amount of bismuth subsalicylate administered in a single dose is of clinical significance because the gastrointestinal tracts of adult humans and dogs absorb different amounts of BSS depending on the physiology and pH of the tract.

**Conclusion:**

We used an external standard technique to calculate the concentration of bismuth subsalicylate in Pepto Bismol. By diluting 12.7035g of Pepto in 250.00mL of $HNO_3$ to create an unknown Bi concentration in a digested suspension, we were able to apply the absorbance to the calibration curve prepared by Bi external standard solutions. The original concentration of bismuth subsalicylate in Pepto-Bismol was determined to be 521.976mg/30mL, which was a 0.576% error from the active ingredient concentration on the label. The data from this experiment suggests that the external standard technique is a fairly accurate way to determine unknown concentrations of compounds in a sample. A potential experiment relating these results to pharmaceutic research could be determining the maximum concentration of BSS in digestive aids for adults, children, and even pets. This would allow for more precise, individualized dose recommendations for sensitive gastrointestinal tracts.

**Refrences:**


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