ANALYSIS OF BISMUTH CONTENT IN A PEPTO-BISMOL TABLETS USING X-RAY FLUORESCENCE SPECTROSCOPY (XRF)

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ABSTRACT

X-ray fluorescence spectroscopy (XRF) was used for the determination of bismuth content in a Pepto-Bismol brand antacid tablet. Three unknowns tablet obtained from the internet purported to be Pepto-Bismol tablet were investigated for bismuth content and the unknown tablet 2 was found to be a counterfeit product due to the absence of bismuth in its spectra during the investigation, in comparison to the official Pepto-Bismol tablet. Standard addition method and external calibration method were utilised for the determination of the amount of bismuth in the official Pepto-Bismol tablet and the mass was found to be 272.46 mg in comparison to the 262.50 mg provided on the box by the manufacturer while 236.46 was found in comparison to the 262.50 using external calibration. XRF technique utilised during this investigation shows many advantages such as high sensitivity, high detection limit, simple preparation method, and excellent reproducibility.

Keywords: XRF, bismuth, counterfeit.

INTRODUCTION

Bismuth is an essential component in therapy utilised to treat gastro-intestinal illness (oligodynamic effects) such as stomach upset, nausea, diarrhoea, heartburn, indigestion, or symptoms associated with drinking or eating too much and is also an essential component in internal deodorants (Gadhari et al, 2010). Bismuth is found in sea water and in human hair. However, high consumption of bismuth in the body can lead to poisoning, mostly affecting the liver and kidney. Overexposure to this element can also result in the formation of black stools, and black tongue on the ginginal, also called bismuth lining (Maretti et al, 1998). Thus, the determination of bismuth at trace amount is crucial for prognostic and prodiagnostic purposes.

Techniques utilised to determine the amount of bismuth comprises of flame atomic absorption spectroscopy (FAAS), inductively coupled plasma-optical emission spectroscopy (ICP-OES),tungsten trap hydride generation atomic absorption spectrometry, atomic fluorescence spectroscopy (AFS) and x-ray fluorescence spectroscopy (XRF). The latter is a powerful analytical spectroscopic method, the technique utilises the x-rays by using them to excite the electrons within the sample of interest (Maretti et al, 1998; Gadhari et al, 2010). These excited electrons leave their associated orbital within the atom and are replaced by a lower energy electron, but the excited will quickly return to their associated ground states by releasing energy in either a non-radiant of a fluorescence manner (Bruker, 2015). Two general setups for XRF equipment includes; energy dispersive x-ray fluorescence spectrometers (ED-XRF) and wavelength dispersive x-ray fluorescence spectrometers (WD-XRF) (Gadhari et al, 2010).

In this study, a rapid, inexpensive, versatile XRF method was utilised to address a range of counterfeit problem in Pepto-Bismol tablet. Owing to the emergence of an antacid drug counterfeiting in West Africa, and Southeast Asia. In these regions, there is growing problem

of counterfeiting of Pepto-Bismol tablet, such counterfeits contain sub therapeutic quantities of bismuth or no bismuth at all, and have resulted in the aforementioned diseases (Degardin et al, 2014; Koesdjojo et al, 2014; USFDA, 2014). Identification of authentic bismuth is therefore essential to prevent fatalities. Detection of bismuth by XRF is proving to be challenging since drift occurs in x-ray sources, and there is also a possibility of matrix effects, the intensity is also affected by particle size (Anderson, 2009; EC, 2014; Chikowe et al, 2015).

The provision of good quality drugs and pharmaceutical preparations represent the backbone of every health care system worldwide; ensuring their availability is crucial for effective treatments and lifesaving therapies (Hollein et al, 2015; Renschler et al, 2015). A Counterfeit drug is a vast global problem. It might lead to death from untreated diseases, reducing confidence in some vital drugs, large economic losses for the legitimate manufacturers and possible drug resistance (Visser et al, 2015; Wang et al, 2015; Taher et al, 2014).

The purpose of the study was to examine three unknown tablets, purported to be antacids similar to Pepto-Bismol, using Twin-X ED-XRD, and comparing them to an official Pepto-Bismol tablet. This is to identify if any of the unknowns is a counterfeit product. This is becoming a severe problem with private companies being able to freely distribute products using the internet. Also again, using the Twin-X equipment, the analysis of Pepto-Bismol tablet will be performed, using both the standard addition and external calibration method. This technique illustrates two different methods in finding an unknown concentration within a sample, the results of which can be directly compared.

MATERIALS AND METHODS

Materials

Genuine Pepto-Bismol tablet was purchased from Boots (Huddersfield, UK). Three unknown tablets purported to be Pepto-Bismol were purchased over the internet. Bismuth subsalicylate (MF: $C_7H_5O_4Bi$, MW: 362.09 $gmol^{-1}$, CAS: 14882-18-9, ASSAY: 99 %), and bismuth (III) oxide (MF: Bi_2O_3, MW: 465.96 $gmol^{-1}$, CAS: 1304-76-3: ASSAY: 99.99 %), were purchased from Sigma-Aldrich (Dorset, UK). Powdered wax and cornflour were purchased from Alibaba, Espanol and Tesco, UK respectively.

Methods

Determination of Bismuth

A Twin X, XRF spectrometer (Oxford instrument) was used to investigate genuine Pepto-Bismol tablet and the three unknown tablets. The spectrometer was programmed to scan low weight and high weight elements (K_α and K_β), the spectra were processed to identify smooth peaks, and the bismuth region was focused on with peaks identified and the spectra generated for later evaluation.

Table 1. Observed physical characteristics of the three unknown tablets that are purportedly Pepto-Bismol alternatives

	Physical Observations	
Tablet	Relative size	Colour
Pepto-Bismol	-	Light pink, few white speckles.
Tablet 1	Equal size	Lighter pink, many white speckles.
Tablet 2	Larger	Medium pink, no speckles.
Tablet 3	Smaller	Dark pink, no speckles.

The table details the unknown tablet's size relative to the official Pepto-Bismol tablet, and also the colour of all the tablet analysed.

Standard addition method

Two tablets (x and y) were made up from powdered wax, cornflour, and Pepto-Bismol tablet, all were weighed by difference on analytical balance (Mettler AT201) to two decimal places (table 2). A standard addition of Bi_2O_3 was added to tablet y. The tablet formulations were stacked on top of each other and loaded into a ball mill, milled for 10 minutes at 350 rpm. The formulations were then pressed into tablets.

Table 2. Measurement of masses (mg) of the excipients used in the formulation of tablet x and y

	Mass Used (mg)	
Ingredient	Tablet x	Tablet y
Pepto-Bismol	490.94	512.41
Powdered wax	487.03	522.65
Cornflour	992.16	869.19
Bismuth (III) oxide	N/A	105.55
Total mass	**1970.13**	**2009.80**

The Pepto-Bismol tablet had a mass of 1098.10 mg and each tablet allegedly contained 262.50 mg of bismuth compound

Table 3. The frequency under the peak (cps) in the bismuth region of interest for the two tablets formulated using the recipes shown in table 3

Sample	Frequency under peak (cps)
Blank	1051
Tablet x	1456
Tablet y	1723

External calibration method

A Twin X, XRF spectrometer (Oxford instrument) was used to determine the peak height (cps) of bismuth standards. The five standards (0, 25, 50, 100, 200 mg of bismuth). The peak height (cps) of each standard including tablet x was detailed in table 4.

Table 4. Peak height (cps) and the amount of bismuth (mg) including tablet x

Amount Bi (mg)	Peak height (cps)
0	10.5
25	20.5
50	28.7
100	40.5
200	59.5
Tablet x	42.9

The data presented in table 4 can also be seen in figure 1

RESULTS AND DISCUSSION

Bismuth contents in Pepto-Bismol and the three unknown tablets

Three unknown tablets were analysed to identify their contents when compared to official Pepto-Bismol tablet. Figure 1 shows the spectra of a genuine Pepto-Bismol tablet. There are peaks for calcium (Ca), iron (Fe), zinc (Zn), multiple peaks for bismuth (Bi) and argon (Ar) having a small peak. The peak intensities are directly proportional to a number of element presents.

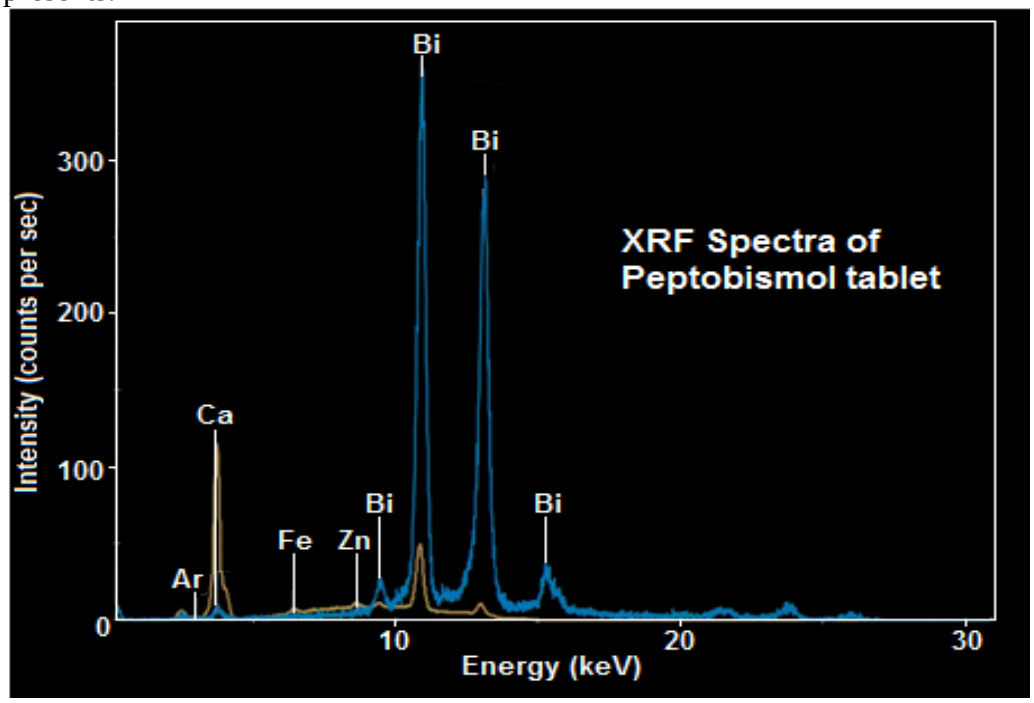

Figure 1. XRF spectra of Pepto-Bismol tablet

The spectrum gained showed a small peak for argon (identified as background interference due to internal XRF target) and large $K_{\alpha 1}$ signals accompanying $K_{\beta 1}$ signals of the aforementioned elements.

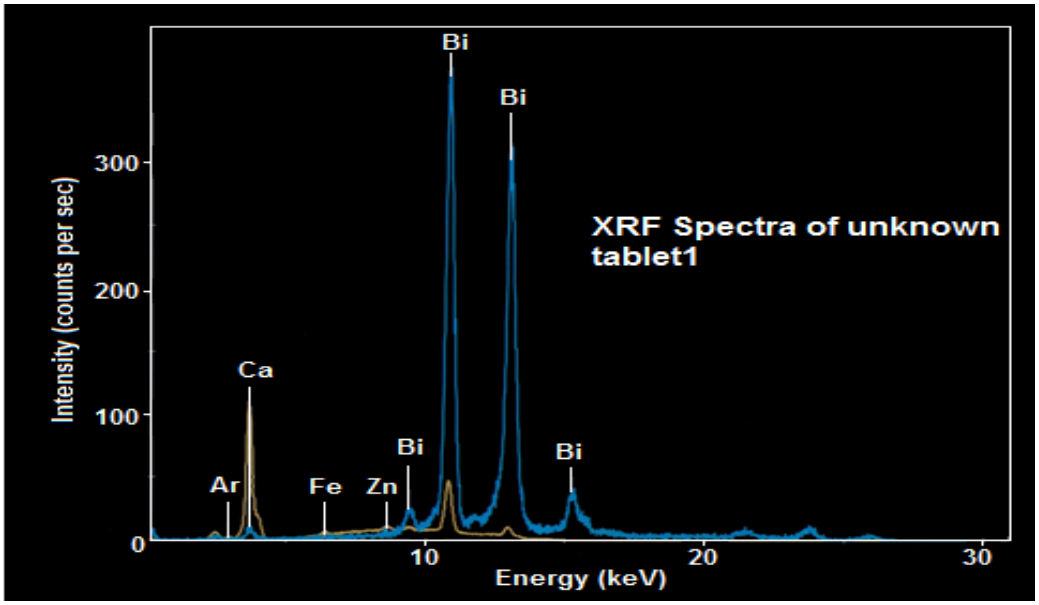

Figure 2. XRF spectra of unknown tablet 1

Comparing Pepto-Bismol tablet and unknown tablet 1, same intensities and peaks for calcium (Ca), zinc (Zn), iron (Fe), and bismuth (Bi) were observed. Tablet 1, therefore, was deduced to contain a very similar set of composition and concentration to the Pepto-Bismol tablet.

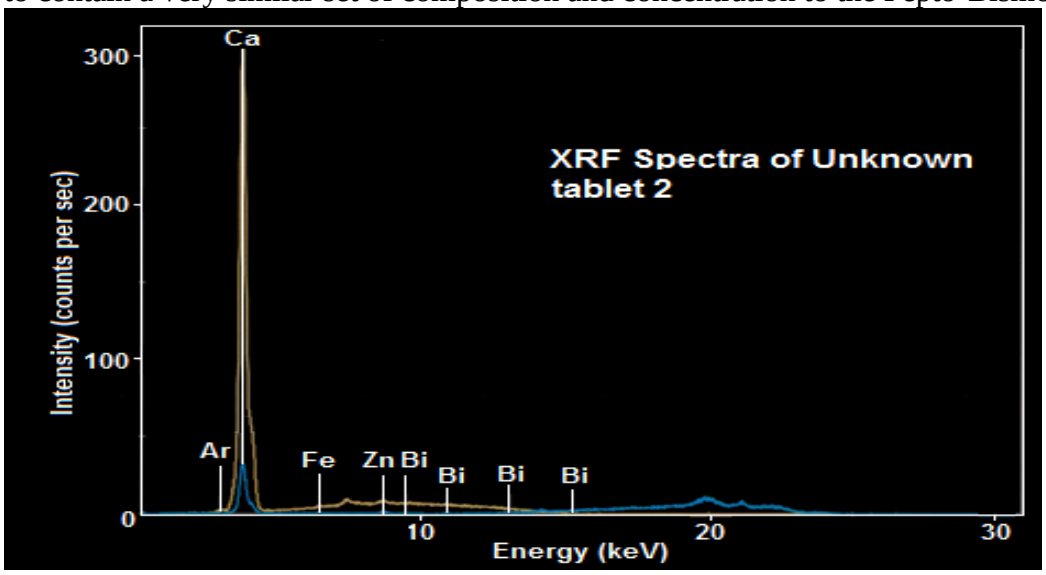

Figure 3. XRF spectra of unknown tablet 2

Figure 3 showed a significantly stronger signal for calcium (Ca), smaller signals for iron (Fe), zinc (Zn), and bismuth (Bi). The intensities are considerably smaller indicating a very low amount of bismuth (Bi).

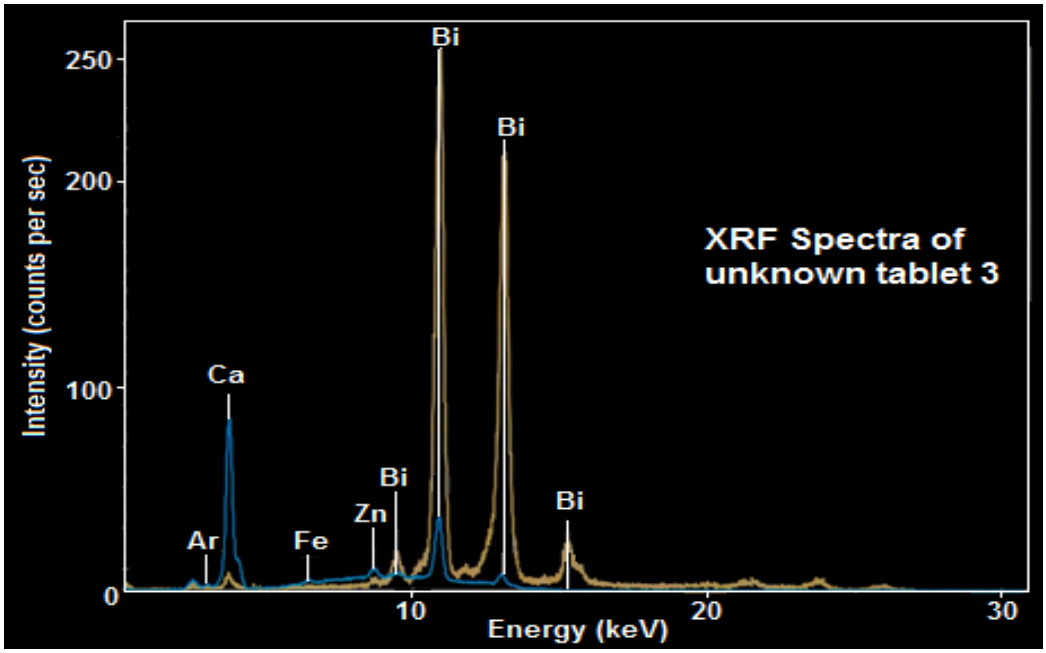

Figure 4. XRF spectra of unknown tablet 3

In figure 4, there are similar peaks for calcium (Ca), zinc (Zn), iron (Fe), and bismuth (Bi). However, the peak intensities are about a third smaller, indicating that this tablet has a similar composition but possibly only two-thirds the bismuth content of a normal Pepto-Bismol tablet.

Method of standard addition

Utilising the results obtained in table 2, it can be seen that the final calculated weight of bismuth subsalicylate found within the official Pepto-Bismol tablet was 272.46 mg. This shows 103 % yield of the literature amount 0f 262.5 mg provided on the box by the manufacturer. Many control measures were utilised in this part of the experiment, ensuring the accuracy of the results.

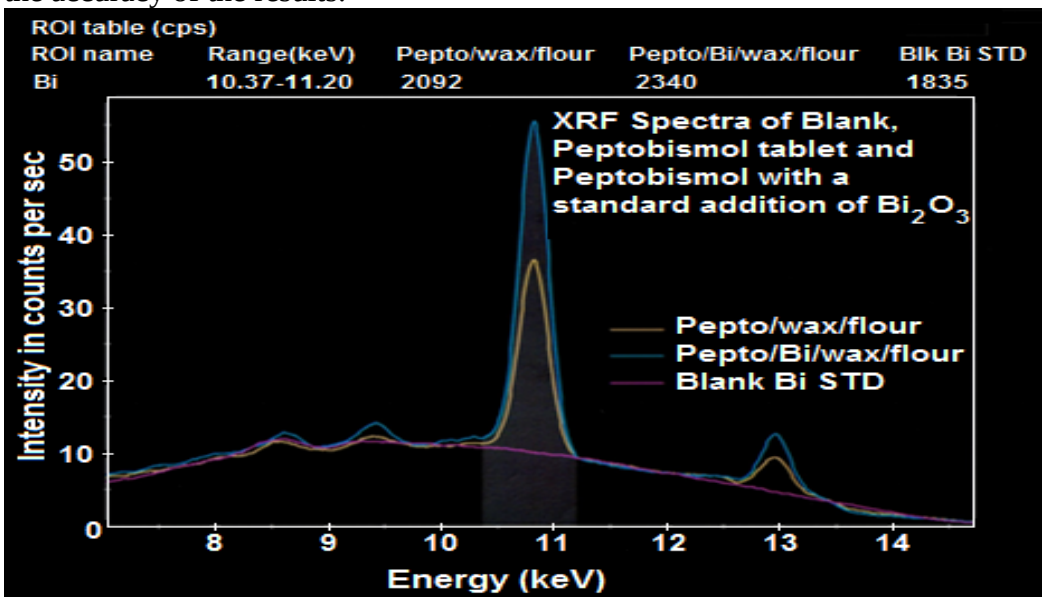

Figure 5. XRF spectra of blank, Pepto-Bismol tablet and Pepto-Bismol with standard addition of Bi_2O_3

Due to the mathematical deduction of the final results, the amount of substance used to formulate the tablet (table 2), did not have to be exactly the same weight as the values suggested in the literature. A long process of sample preparation was adopted. Weighing by difference, using an analytical scale, is an accurate way of knowing the precise amount of substance used in the formulation. This ensures any residue left in the weighing boat is not added to the total weight in the tablet. This technique also eliminates possible bias in the instrument.

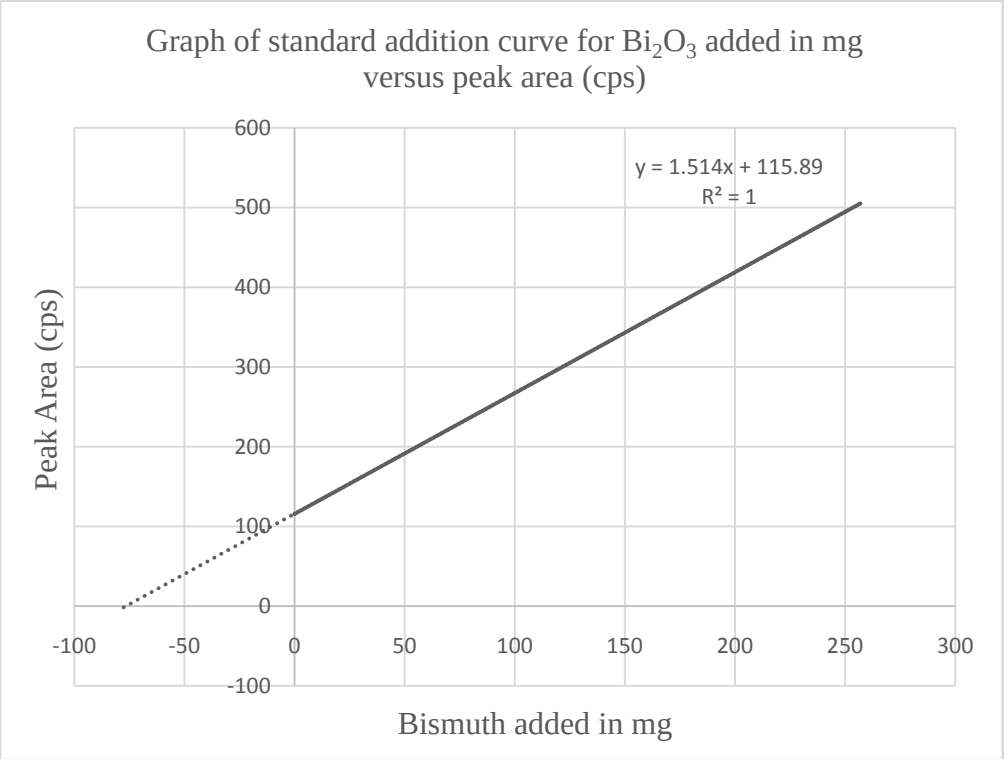

Figure 6. Graph of peak area (cps) versus the amount of bismuth (III) oxide added

The bismuth mass was calculated using the equation of standard addition line. The mass was found to be 76.50 mg bismuth and 132.60 mg bismuth subsalicylate. The method was repeated to test the accuracy and repeatability and the second method yielded the standard addition line equation, y = 1.663x + 115.89. The calculated bismuth mass was 69.70 mg of bismuth and 120.74 mg bismuth subsalicylate.

External Calibration method

The total bismuth subsalicylate amount in the official Pepto-Bismol tablet, using this method, was identifiable as 236.44 mg. This is 90 % of the amount suggested by the manufacturer's literature, which is stated as 262. 50 g. this result was obtained through using the polynomial curve presented in figure 7. The plot of residual (not shown) revealed a clear relationship with an inverse parabolic linearity.

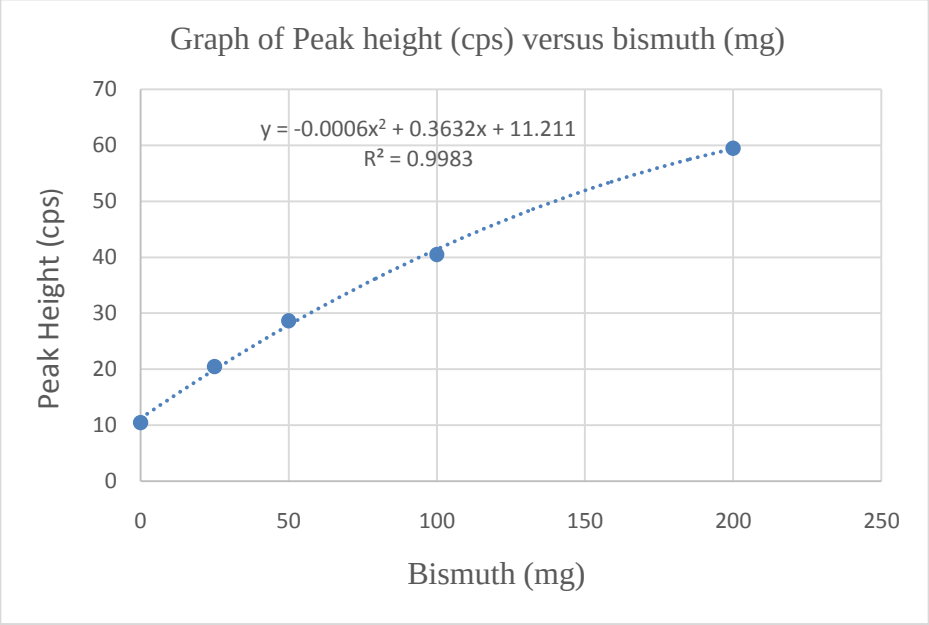

Figure 7. Graph of peak height (cps) versus amount of bismuth (mg)

Using the data gained from the equation of the graph, it can be deduced that the theoretical bismuth subsalicylate observed in Pepto-Bismol was 267.28 mg. This represents 97 % of the manufacturers claimed amount.

CONCLUSION

Counterfeit medicines and efforts to combat their distribution still are a highly critical issue in low and middle-income countries. It is essential to have a constant quality control of pharmaceuticals. Throughout this study examinations have been made to gather several information. In the first objective of the research, the unknown tablet identification, it was clearly observable that tablet 2 was a counterfeit product. This proves that this technique is very useful in quick and accurate clinical drug screening process. In industrial applications this is invaluable due to the rising rate of private drug dispensaries due to the growth in online drug distributers.

In the second part of the study, the concentration of bismuth subsalicylate in Pepto-Bismol was calculated as being 272.46 ± 3.14 mg. The error margin given is relative to one standard deviation and the calculated systematic error of the balanced used. This value represent 97 % of the value quoted by the manufacturer. Utilising the polynomial results of the external calibration method, the concentration of bismuth subsalicylate in Pepto-Bismol was calculated as being 236.44 ± 15.64 mg and 271.28 ± 15.64 mg respectively. The error margin used in these results is relative to the standard error of the straight line generated. The polynomial results represent 90 % of the manufacturers quoted value, and the linear results showed 103 % of the value of subsalicylate.

Owing to the agreement between linear and addition calibration results, it can be assumed that they are more representative of the true value of the bismuth compound amounts found in Pepto-Bismol. The hypothetical lack of errors assumed for the polynomial curve show a clear systematic error in the machine used, or the error in the composition of the standard tablets used in the experiment. This suggests that the external calibration method results in much more significant data being produced from each test, but due to speed and cost of

adopting the standard addition method, coupled with the diminished number of variables, makes standard addition a valuable analytical technique. With both methods providing substantial advantages and disadvantages, and both methods producing significantly similar final results, it is difficult to distinguish which method is better. Due to a more reliable and statistically sound error margin, an external calibration method would be preferable for this type of experiment.

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REFERENCES

1. Anderson, Tatum. "Confusion over counterfeit drugs in Uganda." *The Lancet*373.9681 (2009): 2097-2098.
2. Chikowe, Ibrahim, et al. "Post-marketing surveillance of anti-malarial medicines used in Malawi." *Malaria journal* 14.1 (2015): 127.
3. Dégardin, Klara, Yves Roggo, and Pierre Margot. "Understanding and fighting the medicine counterfeit market." *Journal of pharmaceutical and biomedical analysis* 87 (2014): 167-175.
4. European Commission, Summary of community customs activities on counterfeit and piracy, 2014. Available from: http://ec.europa.eu/taxation_customs/resources/documents/customs/customs_controls/
5. Gadhari, Nayan S., et al. "Potentiometric stripping analysis of bismuth based on carbon paste electrode modified with cryptand [2.2. 1] and multiwalled carbon nanotubes." *ElectrochimicaActa* 56.2 (2010): 627-635.
6. Höllein, Ludwig, et al. "Routine quality control of medicines in developing countries: analytical challenges, regulatory infrastructures and the prevalence of counterfeit medicines in tanzania." *TrAC Trends in Analytical Chemistry* (2015).
7. Koesdjojo, Myra T., et al. "Low-cost, high-speed identification of counterfeit antimalarial drugs on paper." *Talanta* 130 (2014): 122-127.
8. Lu, Feng, et al. "A novel identification system for counterfeit drugs based on portable Raman spectroscopy." *Chemometrics and Intelligent Laboratory Systems* 127 (2013): 63-69.
9. Maretti, M.I., Bueno, S., & do Amarel, L.C. (1998). X-ray fluorescence determination of adsorbed copper on activated charcoal after glycerin complexation. *Química Nova.21* (4), 13083-13970.
10. Renschler, John P., et al. "Estimated Under-Five Deaths Associated with Poor-Quality Antimalarials in Sub-Saharan Africa." *The American journal of tropical medicine and hygiene* (2015): 14-0725.
11. Taher, Mohammad Ali, Mina Rahimi, and Hamid Fazelirad. "A sensitive fluorescence quenching method for determination of bismuth with tiron."*Journal of Luminescence* 145 (2014): 976-980.
12. U.S. Food and Drug Administration, Counterfeit version of avastin in U.S. distribution, 2014. Available from: http://www.fda.gov/Drugs/DrugSafety/ucm291960.htm (accessed 23.05.14).

13. Visser, Benjamin J., et al. "Assessing the quality of anti-malarial drugs from Gabonese pharmacies using the MiniLab®: a field study." *Malaria journal*14.1 (2015): 273.
14. Wang, Ting, et al. "Quality of antiretroviral and opportunistic infection medications dispensed from developing countries and Internet pharmacies."*Journal of clinical pharmacy and therapeutics* 40.1 (2015): 68-75.