Analysis of an OTC Multivitamin Tablet Using X-ray Fluorescence Spectroscopy and Atomic Absorption Spectroscopy

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Abstract: The elemental composition of a branded OTC multivitamin tablet was determined using the spectroscopic methods: atomic absorption spectroscopy (AAS), energy dispersive x-ray fluorescence spectroscopy (EDXRFS) and wave dispersive x-ray fluorescence spectroscopy (WDXRFS). Tablets were randomly chosen from the same batch and their elemental composition was expressed in terms of Ca, Cu and Fe. The quantitative analysis was performed using standard addition method for AAS and standard-less method for both XRFS methods. The elemental composition of the tablet obtained from AAS measurements showed that it contains: 257.9mg ± 5.5 for Ca, 1.972 mg ± 0.090 for Cu and 64.46 mg ± 1.73 for Fe. The analysis of the composition of the tablet obtained using EDXRF and WDXRF was reported in terms of % metal oxide. The semi quantitative standard-less EQUA-ALL method was used for the S2 Ranger EDXRFS and results showed that the tablet contains 28.2%, 7.52% and 0.134% of CaO, Fe_2O_3 and CuO respectively for the loose powder form while 33.7%, 9.27% and 0.179% of CaO, Fe_2O_3 and CuO respectively for the pressed pellet form. On the other hand, the composition of the tablet obtained with the use of a standard-less method for S8 Tiger WDXRFS gave the following results: 26.46% CaO, 6.236% Fe_2O_3 and 0.0455% CuO from the analysis of the loose powder form and 27.87% CaO, 6.741% Fe_2O_3 and 0.0494% CuO for the pressed pellet form. Although the use of XRF is simple and boasts of the use of the standardless method, the reproducibility of the results gathered from XRFS, as represented by the related methods of EDXRF and WDXRF were inferior compared to the results obtained using AAS.

Key Words: AAS;, XRF; standardless method; EDXRF; WDXRF; analysis of multivitamin tablet

1. INTRODUCTION

The most common analytical technique used for the determination of the elemental composition of various samples is the atomic absorption spectroscopy (AAS). Standard analytical methods and procedures involving the use of AAS for the determination of metal contents has been tested and established. However, this method requires tedious sample preparation and standardization process. The method involved in the sample preparation increases the risk to personal and systematic errors and the use of concentrated acids for sample digestion makes the method hazardous to the environment.

X-ray fluorescence spectroscopy (XRFS) is a new analytical technique designed to analyze elements present in a sample using x-ray as the source of radiation. XRFS was found to be a faster, more practical technique used in analyzing elements due to its non-destructive sample preparation process. It can analyze different types of samples in a shorter span of time. Unlike AAS, it does not require the use of separate hollow cathode lamps as source of radiation and thus XRFS can analyze a wider range of elements.

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Due to the non-destructive nature of XRFS, several studies have been conducted to evaluate its efficiency on various analyses of different materials. A study by Richard King proposed the use XRFS for mass analysis of various food samples highlighting the ease of sample preparation, faster running time and efficient results with comparison to the results obtained by standard chemical methods. Also, he states that XRFS is more preferred for mass analysis of samples because of its easy sample preparation and faster running time.[i] Perring et al. recommended that EDXRF can be used as a quick reliable method for the quantification of iron, copper, and zinc in food premixes. They state that good calibrations should be done in order to produce efficient results.[ii]

XRFS has also been used in determining the quality of rice through quantitative determination of elements such as copper, iron, zinc, cadmium, and vitamin A. It was stated that XRFS was better to use since it does not require sample digestion. The results were found to be precise and reproducible.[iii] In Japan, cadmium pollution in rice has become a widespread problem. A quick way to analyze trace amount of cadmium in brown rice using high energy dispersive XRF with three-dimensional polarization optics was developed by Nagayama, H. Their results exhibited a linear correlation of 0.023 to 1.82 ppm with the certified reference materials. They had successfully determined cadmium concentration in rice at 20ppb using Cd K. ALPHA line after optimizing the optics together with the secondary target.[iv]

The most prominent application of XRFS is in heavy metal analysis especially in water samples. Moriyama, T. used XRFS to analyze lead and arsenic in various river water samples. He preferred the use of XRFS since it is simpler, rapid and reproducible in comparison with inductively coupled plasma mass spectroscopy and AAS.[v] Melquiades, F. and Appolono C. proposed that XRFS can be used as an alternative technique in analyzing contamination in water samples. They compared XRFS with atomic absorption spectroscopy and inductively coupled plasma atomic emission spectrometry (ICP-AES) in terms of rapidness of analysis, precision, reliability, accuracy and low detection limits for trace elements. They used a field portable XRFS since it uses an efficient radioisotope source excitation and has highly sensitive detectors. They claim that this method is more cost effective and time efficient for on-site analysis.[vi]

There are fourteen (14) essential minerals which are crucial to one's growth and development. These minerals are calcium (Ca), chromium (Cr), copper (Cu), fluorine (F), iodine (I), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), phosphorus (P), potassium (K), selenium (Se), sodium (Na) and zinc (Zn). Too little amounts of these essential minerals can cause deficiency diseases while an excess of these minerals can also be toxic.

This study compares the quantitative elemental analysis of an over the counter (OTC) multivitamin tablet for pregnant women using XRFS and AAS based on reproducibility of results, ease of sample preparation and level of support and training required in running the equipment. It aims to conduct a quantitative analysis of the mineral content of the OTC multivitamin sample by measuring the amount of calcium, copper and iron present per tablet using XRFS and AAS. The reproducibility of the results and method will be evaluated by comparing the resulting data obtained from both instrumental techniques.

Due to the specific mineral requirement of the human body, multivitamins are properly labeled according to its composition. The results obtained from this study will confirm the mineral composition of the multivitamin sample as stated in its label. The study also aims to validate the use of XRFS as an alternative analytical technique for elemental analysis.

The study focused on the determination of calcium (Ca), copper (Cu) and iron (Fe) present in a branded OTC multivitamin tablet for pregnant women using Energy Dispersive XRF (EDXRF), Wavelength Dispersive XRF (WDXRF) and Atomic Absorption Spectroscopy. Both loose powder and pressed pellet sampling methods were used for measurements using the EDXRF and WDXRF while the standard addition method was used for AAS measurements.

2. METHODOLOGY

The samples used for this study is a branded OTC multivitamin tablet for pregnant women purchased from a local pharmacy in Manila with the same batch number. All chemicals and reagents were analytical reagent grade and did not require any further purification prior to use.

Measurements were conducted using the instruments Varian SpectrAA 200 Atomic Absorption Spectrometer, S2 Ranger Energy Dispersive X-ray Fluorescence Spectrometer and S8

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Tiger 1K Wavelength-Dispersive X-ray Fluorescence Spectrometer.

2.1 Quantitative Analysis of Ca, Cu and Fe Using Atomic Absorption Spectroscopy (AAS)

Each tablet was accurately weighed, pulverized and digested in hot concentrated nitric acid. A 1.000 L dilute sample solution (DSS) was prepared from each homogenized sample. Standard solutions of Cu, Ca and Fe were prepared by diluting the commercially available 1000 ppm standard solutions (Atomic Absorption Standard Grade). The concentration of the dilute solutions was based on the working range for each element. The quantitative analysis of the multivitamin was conducted using the standard addition method. The instrumental parameters of the AAS for each element are listed in Table 1.

Table 1. List of Instrument Parameters Used for the AAS Measurements

Element: Calcium (Ca)

Working Range (µg/mL)	2-800
Wavelength (nm)	422.7
Slit Width (µg/mL)	0.5

Element: Copper (Cu)

Working Range (µg/mL)	1-280
Wavelength (nm)	327.4
Slit Width (µg/mL)	0.2

Element: Iron (Fe)

Working Range (µg/mL)	1-100
Wavelength (nm)	372.0
Slit Width (µg/mL)	0.2

2.2 Quantitative Analysis of Ca, Cu and Fe Using X-Ray Fluorescence (XRF) Spectroscopy

The sample preparation and analytical measurements were performed in Bruker Laboratories (Bruker AXS GmbH) Karlsruhe Germany.

The measurements were conducted using two sampling methods namely, as loose powder and as pressed pellet. The samples were prepared by pulverizing eight (8) tablets of the multivitamin sample using an agate mortar and pestle. Measurement as loose powder was done by placing seven (7) grams of the pulverized sample into a liquid cup containing a 4µm Prolene film at the bottom. The pulverized sample was compacted by carefully knocking the cup on top of a table. The loose powder sample was measured in helium atmosphere.

For pressed pellet method, 10 grams of the pulverized sample was weighed and pressed into a pellet (diameter = 40mm) by the application of 20 tons of force for 30 seconds. The measurements were conducted under vacuum.

2.2.1 Energy Dispersive X-Ray Fluorescence (EDXRF) Spectroscopic Analysis

The samples were analyzed using a semi-quantitative software called EQUA-ALL which is a unique universal calibration that automatically corrects line overlaps without the need for any adjustment. The elements were measured in three different conditions for light, medium and heavy weight elements. Table 2 contains a list of the parameters used for the EDXRF Spectroscopic measurements.

Table 2. List of the Instrumental Parameters Used for EDXRF Spectroscopic Measurements

Light Elements (Voltage = 20 kV)

Filter (µm)	none
Measurement Time (s)	35
Current (µA)	Optimized
Maximum count rate (kcps)	100

Medium Elements (Voltage = 40 kV)

Filter (µm)	Al 500
Measurement Time (s)	35
Current (µA)	Optimized
Maximum count rate (kcps)	100

Heavy Elements (Voltage = 50 kV)

Filter (µm)	Cu-250
Measurement Time (s)	100
Current (µA)	Optimized
Maximum count rate (kcps)	100

2.2.2 Wavelength Dispersive X-Ray Fluorescence (WDXRF) Spectroscopic Analysis

The samples were analyzed using a standard-less method and were directly subjected to the machine for analysis. No details on the instrument parameters were provided.

3. RESULTS AND DISCUSSION

Representative tablets of the branded OTC multivitamin tablet were randomly chosen from the same batch and were analyzed for its elemental and quantitative composition specifically Ca, Cu and Fe using the following instrumentation techniques: the standard addition method for AAS, the semi quantitative standard-less EQUA-ALL method for S2 Ranger EDXRF and a standard-less method for S8 Tiger WDXRF.

The multivitamin label states that each tablet contains 250mg calcium, 2.0mg copper and 60mg iron in the form of calcium carbonate, cupric oxide and ferrous fumarate respectively. These values will be the basis of comparison for the results that will be generated from the analysis.

3.1 Results of the Elemental Analysis using AAS

The absorbance of the spiked sample solutions were plotted as a function of the concentrations of the added standard solution of the element being analyzed. The equation of the calibration curve was determined using the calibration model, least squares method. From the x-intercept of the equation, the concentration of the element per sample was determined and the corresponding amount in grams was calculated.

Only the plots of the absorbance vs. concentration of the standard solution of the elements Cu, Ca and Fe added into aliquot portions of the dilute sample solution containing the tablet No. 2 are shown in Figures 1, 2 and 3 respectively. The results of the elemental analysis for each element are summarized and presented in Table Nos. 3-5
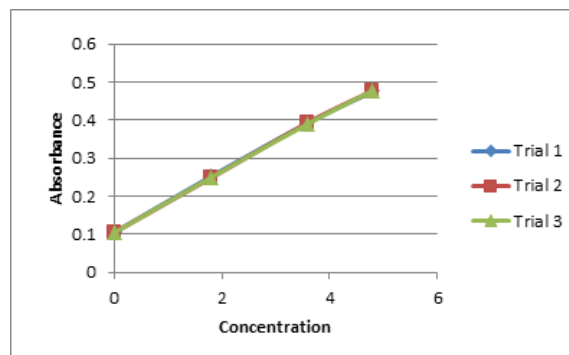

Figure 1. Quantitative Analysis of Cu Using the Standard Addition Method (Tablet No. 2)

Table 3. Summary of the Quantitative Analysis of Copper (Cu) Using AAS Measurements

	Tablet 1	Tablet 2	Tablet 3
Trial 1	2.114	2.010	1.866
Trial 2	2.084	1.943	1.919
Trial 3		1.950	1.891
Average	2.099±0.021	1.968±0.037	1.892±0.027
Total average		1.972 ± 0.090	

According to Table 3, the average composition of Cu present in the tablet is 1.972 mg ± 0.090 with only 1.39% difference from the stated value of the multivitamin in the label.
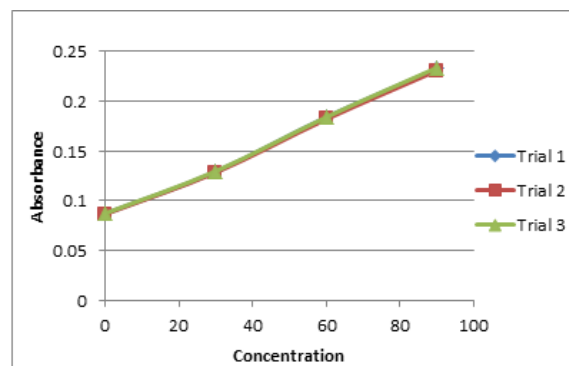

Figure 2. Quantitative Analysis of Ca Using the Standard Addition Method (Tablet No. 2)

Table 4. Summary of the Quantitative Analysis of Calcium (Ca) Using AAS Measurements

	Tablet 1	Tablet 2	Tablet 3
Trial 1	247.2	259.1	260.9
Trial 2	250.0	260.8	259.5
Trial 3	260.6	258.9	264.2
Average	252.6±7.1	259.6±1.0	261.5±2.4
Total average		257.9 ± 5.5	

Based on the summary of the results presented in Table 4, with the use of the standard addition method for AAS, it revealed that the average amount of Ca present in the sample is 257.9mg ± 5.5 with a 3.16% difference from the values indicated in the label.
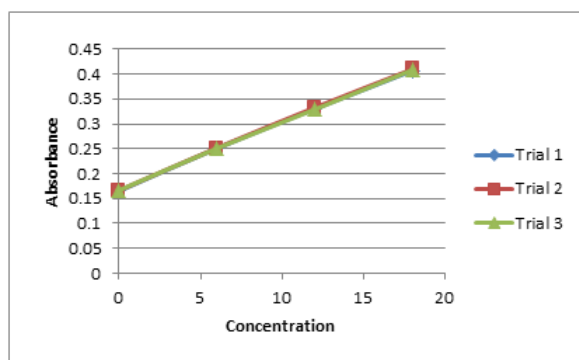

Figure 3. Quantitative Analysis of Fe Using the Standard A

Table 5 Summary of the Quantitative Analysis of Iron (Fe)

	Tablet 1	Tablet 2	Tablet 3
Trial 1	65.63	62.52	63.47
Trial 2	64.75	62.27	65.81
Trial 3	67.07	62.85	65.76
Average	65.82±1.17	62.55±0.29	65.01±1.34
Total average		64.46 ± 1.73	

The evaluation of the AAS measurements of the iron content revealed that the amount of Fe present in the sample is 64.46mg ± 1.73 with a 7.43% difference from the values indicated in the label.

In general, a comparison of the values representing the chemical composition of the tablet showed a good agreement between the specifications of the metals on the label and actual values obtained.

3.2 Results of the Elemental Analysis using EDXRF and WDXRF

The results for the two XRFS techniques were reported in terms of the percentage of the oxides of Calcium (Ca), Copper (Cu) and Iron (Fe) in the form of Calcium Oxide (CaO), Cupric Oxide (CuO) and Ferric Oxide (Fe_2O_3) respectively.

Table 6. Results of the EDXRF (S2 Ranger) Analysis of the of the OTC Multivitamin Tablet

	Loose Powder	Pressed Pellet
[%]	7 g in liquid cup	10 g
CaO	28.2	33.7
Fe_2O_3	7.52	9.27
CuO	0.134	0.179

Table 7 Results of the WDXRF (S8 Tiger) Analysis of the OTC Multivitamin Tablet

	Loose Powder	Pressed Pellet
[%]	7g liquid cup	10 g
CaO	26.46	27.87
Fe_2O_3	6.236	6.741
CuO	0.0455	0.0494

The WDXRF results for CaO and Fe_2O_3 show a close comparison with the AAS results and the label stated by the multivitamin brand with percent differences ranging from 2% to 10% only unlike those of the EDXRF which varied from 4% to 34% difference. While for CuO, more than 70% difference was calculated. This was probably due to the effect of a secondary enhancement where the x-ray emitted by a heavier element stimulates the x-ray emissions of a lighter element.

4. CONCLUSION

The quantitative analysis of a branded OTC multivitamin tablet designed for pregnant women was performed using AAS, EDXRF and WDXRF. Results obtained from AAS

measurements reflect a high degree of reproducibility and the values obtained are consistent with those reported from the product label. The AAS results showed agreeable values with the label of the multivitamin sample stating the amount of elements present per tablet. The resulting amount per tablet was 257.9mg±5.5 for Ca, 1.972mg±0.090 for Cu and 64.46mg±1.73 for Fe. The XRF allows the analysis of a wide range of elements and does not involve a complicated sample preparation.

However, results showed that significant deviation of the reported values was obtained using XRFS. Furthermore, a comparison of the values for WDXRF and EDXRF lack agreement in results. These uncertainties may due to the difference in the sample matrix of the tablet and the reference used in the preparation of the reference calibration curve and due to secondary enhancements brought about by heavier elements present in the sample.

The XRFS as analytical tool is better suited for qualitative analysis unless a suitable blank that has identical properties as the sample matrix of the sample is available. This is also the reason why, the use of the standard-less method is not highly recommended for the analysis of samples that have varied composition.

A comparison of the EDXRF and WDXRF reveal that WDXRF was more accurate in elemental analysis since it presented closer values between the loose powder and pressed pellet form. Also, it has a higher resolution and higher count rates with the same standard-less and non-destructive approach to samples.

Despite the high agreement of the results obtained using AAS, it is noted that sample preparation was a big concern for the analyst. The use of large amounts of acids and standard solutions introduces wastes to the environment and is a cause for concern.

5. ACKNOWLEDGEMENT

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