EVALUATION OF THE QUALITY OF DIFFERENT BRANDS OF ASCORBIC ACID IN FREETOWN, SIERRA LEONE

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ABSTRACT

Objective: This study evaluates the registration status and the quality of nine brands of Vitamin C sold in Freetown, Sierra Leone, using official and non-official methods of analysis.

Methods: The parameters taken into consideration during the study include non-official methods (friability and disintegration) and the official methods (identification test, uniformity of dosage unit, and assay by titrimetric and colorimetric tests). The different brands of vitamin C were selected and classified as registered and unregistered based on the data derived from the National Medicines Regulatory Authority.

Results: One vitamin C product collected was unregistered at the National medicines regulatory authority (Pharmacy Board of Sierra Leone). All nine vitamin C products met the requirements for uniformity of dosage unit. The vitamin C products also conformed to the BP specification for friability and identification test (retention factor). The identification test showed that each brand contained vitamin C (ascorbic acid). The disintegration test was not applicable for chewable tablets. Three of the four non-chewable vitamin C products complied with the BP specification for disintegration, while one failed and did not meet the requirement. The assay results for ascorbic acid using titration and colourimetric method were comparable and appropriate for determining vitamin C. Out of a total of nine samples; seven met the requirement for BP specification (Passed) for assay. In contrast, one product did not meet the BP requirement (failed), and one sample had more vitamin C than the Pharmacopoeia limit.

Conclusion: Periodic quality evaluation and routine checks for pharmaceutical products can ascertain the quality of products, their storage conditions and identify potential counterfeit medications.

Keywords: Post-market surveillance, Quality control, Vitamin C, Counterfeit, Quality assurance, Unregistered

INTRODUCTION

Vitamin C or L-ascorbate is a water-soluble vitamin. It is a vital antioxidant that plays an essential role in human nutrition [1]. It acts as a cofactor for some biologically active radicals. Vitamin C is necessary for building blood vessels, ligaments, tendons, bones, and is also essential in synthesizing carnitine, which is involved in metabolism in most plants and some bacteria [1, 2].

WHO suggested that most drugs in the world today are either falsified or substandard and that 10% are from low and middle-income countries. Substandard medicines may come from poor manufacturing and quality assurance processes and reach the public due to lax control measures, whereas falsified medicines are deliberately and fraudulently labelled [3].

Ascorbic acid degrades in the presence of enzymes and atmospheric oxygen [4]. Vitamin C bears an obvious similarity with hexose sugars; hence it is conceivable that the molecule might serve as a carbon source for respiration or bacteria growth that might be fermented [5]. Storage condition of low temperature and humidity retards microbial growth whereby chemical and biological processes are slowed down [6]. However, breaching the protective barrier by microbial growth results in the subsequent destruction of the commodity.

An excellent quality control system can create the platform for employers and employees to develop a fantastic working atmosphere and efficiency resulting from improved wellbeing and quality of life achieved by preventing absence from work and reducing hospital stay. This study adds to research evidence in Sierra Leone and the African region by providing information for researchers and the public on the quality of vitamin C products circulating in Sierra Leone. It also includes rudimentary information on the registration status of the sampled vitamin C products.

MATERIALS AND METHODS

Experimental design

The convenience sampling of nine (9) brands of vitamin C, purchased and collected from Pharmacies in Freetown from the 20th February to the 22nd March 2019 and stored using the recommendations on each label claim. The physicochemical analysis at the National Pharmaceutical Quality Control Laboratory of the Pharmacy Board of Sierra Leone, New England Ville, Freetown, took place from the 20th to the 2nd June 2019.

Test solution, solvent, and reagents

0.1M Ammonium cerium (IV) Sulphate, 1M \( H_2SO_4 \), water, ferroin solution, ethanol, ferricyanide solution.

Instrument used

The instrument includes Analytical balance, volumetric Flask, friabulator, vernier calliper, filter paper, separating funnel, pipette, mortar, pestle, weighing boat, timer, measuring cylinder, burette, burette stand, UV lamp, UV apparatus, Thin layer chromatography chamber, Thin layer Chromatography plate, chromatogram.

Test methods used

Active substance identification test

Thin Layer Chromatography Identification: Thin layer Chromatogram of pure vitamin C (0.5%) in water and equivalents solutions of tablets obtained with Ethanol-EtOH: Water-H_2O (6:1) as a solvent system using their Rf values and colour characteristics under ultraviolet light [2-7].

Uniformity of dosage units

Weighed ten tablets individually using an analytical balance. The weight of the ten (10) tablets is determined to calculate the average
weight. The assay value for each tablet was determined using the average weight. The acceptance criteria were determined using the method in the British Pharmacopoeia 2018 [7].

**Disintegration test**

The requirement for disintegration does not apply to Chewable Ascorbic Acid Tablet. One dosage unit was placed in each of the basket’s six tubes and added a disc. The apparatus, operated using the specified medium of 37 °C as the immersion fluid. The time taken for each of the four tablets of each brand to disintegrate was recorded [7].

**Friability**

Twenty tablets were weighed and subjected to abrasion for 4 min at 100 rev/min using Erweka single drum friability tester. The end of the revolution was then followed by reweighing the twenty tablets to calculate the percentage loss.

If cracked, cleaved, or broken tablets are present in the tablet sample after tumbling, it fails the test. If the results are difficult to interpret or the weight loss is greater than the targeted value, the test procedure was rechecked and repeated, and the mean of three tests, determined. A maximum weight loss (obtained from a single test or the average of three tests) of not more than 1.0% is considered acceptable for most products.

**RESULTS**

**Profile of vitamin C tablets**

For each brand of Vitamin C tablets, a tablet sample was carefully dusted, weighed (W1), and placed in the friabulator at 100rpm (rounds per minute) for four minutes. At the end of the four minutes, the tablets are removed from the drum, dusted, and accurately weighed for the second time (W2) [7].

Percentage of Friability of ascorbic acid was determined using the formula below:

\[
\text{Percentage Friability} = \frac{(\text{Initial weight (W1)} - \text{Final weight (W2)})}{\text{Initial weight (W1)}} \times 100
\]

**Assay by titrimetry**

Twenty tablets are weighed together and powdered. A quantity containing 0.15g of ascorbic acid was dissolved in a mixture of 30 ml of water and 20 ml of 1M Sulphuric acid and titrated with 0.1M Ammonium Cerium (IV) Sulphate, using ferroin solution as the indicator [7].

**Assay by colourimetric method**

The absorbance of the coloured complex formed between potassium ferricyanide solution and ascorbic acid measured at 520 nm was used to plot a calibration curve against the concentration of ascorbic acid (2.5-25ug/ml). The amount of ascorbic acid was determined using the regression equation from the calibration curve [2].

| Sample code | Product description | Primary package | Secondary package | Manufacturing country | Storage condition | Label claim | Registration status |
|-------------|---------------------|-----------------|-------------------|----------------------|------------------|-------------|-------------------|
| 001         | Orange cylindrical tablet with a line on the centre of one side | Thermoformed aluminium blister pack of 10tabs | Red and green card box | India | Store at temperature below 25 °C protected from light and moisture | 100 mg | Registered |
| 002         | Orange flavour oval tablets | Orange and white Plastic container | Orange and white Plastic container | United Kingdom | Store in a cool, dry place | 500 mg | Registered |
| 003         | Orange cylindrical tablet with a line on the centre of one side with an inscription (ECL) | Thermoformed aluminium blister pack | Orange and green rectangular card box | Nigeria | Store in a cool, dry place below 30 °C | 100 mg | Registered |
| 004         | Orange cylindrical tablet with an inscription (ECL) | Thermoformed aluminium strip pack | Orange and green card box | Nigeria | Store in a cool, dry place protected from light | 100 mg | Registered |
| 005         | Brown cylindrical tablet | Orange white, and blue plastic container | Orange white, and blue plastic container | USA | Store at room temperature, tightly closed | 500 mg | Registered |
| 006         | Yellow cylindrical tablet with a line on the centre of one side | Orange white, and blue plastic container | Orange and grey plastic container | China | Store in a cool, dry place. Keep out of reach of children | 100 mg | Registered |
| 007         | Lemon yellow cylindrical tablet | Green and white plastic container | Green and white plastic container | China | Store in a cool, dry place below 30 °C | 250 mg | Unregistered |
| 008         | Orange cylindrical tablet | Green and orange plastic container | Green and orange plastic container | Nigeria | Store in a cool and dry place | 100 mg | Registered |
| 009         | Orange and brown tablets | Thermoformed aluminium blister packs | Green and yellow card box | Nigeria | Store in a cool, dry place below 25 °C. Protect from light and moisture and keep out of reach of children. | 100 mg | Unregistered |
Mean, standard deviation and acceptance value of vitamin C

Table 2: Uniformity of dosage units this shows the deviation and acceptance value for each sample of ascorbic acid tablets. Sample 002 did not meet the requirement for acceptance value

| Sample | Mean±SD1 | Calculated acceptance value | Acceptance limits (L1) | Mean±SD2 | Calculated acceptance value | Acceptance limits (L2); lower, upper limit |
|--------|----------|-----------------------------|------------------------|----------|-----------------------------|---------------------------------------------|
| 001    | 106.3±1.70 | 8.94                        | ≤15                    | NA       | 115.06±0.81                | 15.18                                      |
| 002    | 115.1±1.03 | 16.04                       | ≤15                    | 115.06±0.81 | 15.18                       | ≤25; 86.30,143.83                           |
| 003    | 100.9±0.48 | 1.72                        | ≤15                    | NA       | NA                         | NA                                          |
| 004    | 101.6±1.80 | 4.48                        | ≤15                    | NA       | NA                         | NA                                          |
| 005    | 102.7±1.78 | 5.418                       | ≤15                    | NA       | NA                         | NA                                          |
| 006    | 100.4±2.32 | 6.698                       | ≤15                    | NA       | NA                         | NA                                          |
| 007    | 100.2±1.30 | 3.742                       | ≤15                    | NA       | NA                         | NA                                          |
| 008    | 52.6±1.09  | 2.568                       | ≤15                    | NA       | NA                         | NA                                          |
| 009    | 101.5±3.48 | 9.623                       | ≤15                    | NA       | NA                         | NA                                          |

NA: Not Applicable; ne (number of experiments for each sample) = 10; SD = standard deviation

Identification and friability test for vitamin C

Table 2: Identification and Friability test for vitamin C the samples showed the same retention factor (Rf) for each brand of ascorbic acid. Friability percentage calculated to determine the physical strength of compressed and uncoated tablets showed that each tablet had less than 1 % breakage

| Sample | Friability (%)±SD | Rf Values |
|--------|-------------------|-----------|
| 001    | 99.85±0.01        | 0.7       |
| 002    | 99.75±0.05        | 0.7       |
| 003    | 99.73±0.05        | 0.7       |
| 004    | 99.69±0.07        | 0.7       |
| 005    | 100±0.35          | 0.7       |
| 006    | 99.91±0.02        | 0.7       |
| 007    | 98.2±0.30         | 0.7       |
| 008    | 99.3±0.05         | 0.7       |
| 009    | 99.6±0.03         | 0.7       |

NA: Not Applicable; ne (number of experiments for each sample) = 3; SD = standard deviation

Disintegration test for vitamin C

Table 3: Disintegration test for non-chewable vitamin C tablet, the requirement for disintegration does not apply to chewable tablets. All samples tested met the requirement for disintegration, while sample 003 did not comply with the requirement for disintegration as per the British pharmacopoeia

| Sample code | Time (min) | Limit                        |
|-------------|------------|------------------------------|
| 001         | NA         | Not Applicable for Chewable Tablets |
| 002         | NA         | Not Applicable for Chewable Tablets |
| 003         | 30         | <15 min                      |
| 004         | NA         | Not Applicable for Chewable Tablets |
| 005         | NA         | Not Applicable for Chewable Tablets |
| 006         | NA         | Not Applicable for Chewable Tablets |
| 007         | 5          | <15 min                      |
| 008         | 6          | <15 min                      |
| 009         | 7          | <15 min                      |

NA: Not applicable

Assay of vitamin C

Table 4: Assay of vitamin C tablet, six samples (003, 004, 005, 006, 007, and 009) met the requirement for BP pharmacopoeia, while two samples (001 and 002) recorded more significant quantities of vitamin C for one or both quality control analysis (titrimetry and colourimetry) and one sample (008) failed quality control analysis

| Sample code | Titration value (%) | Colorimetric (%) | Limit (%) |
|-------------|---------------------|-----------------|-----------|
| 001         | 106.27              | 11.00           | 95.00-107.50 |
| 002         | 115.07              | >125.00         | 95.00-107.50 |
| 003         | 100.74              | 95.00           | 95.00-107.50 |
| 004         | 101.56              | 100.00          | 95.00-107.50 |
| 005         | 103.40              | 106.25          | 95.00-107.50 |
| 006         | 100.13              | 100.00          | 95.00-107.50 |
| 007         | 100.74              | 103.75          | 95.00-107.50 |
| 008         | 52.42               | 47.50           | 95.00-107.50 |
| 009         | 102.99              | 100.00          | 95.00-107.50 |
DISCUSSION

This study evaluates the different brands of vitamin C (ascorbic acid) in Freetown using the following methods: thin layer chromatography test, uniformity of dosage unit, assay to determine the content of active pharmaceutical ingredients in the pharmaceutical tablet, colourimetric method, friability, and disintegration test [8].

The sample profile in table 1 describes the different brands of Vitamin C based on packaging, manufacturing country, storage condition, and the strength of ascorbic acid used. The uniformity of the dosage unit [8] for determining the consistency in the amount of ascorbic acid for each brand of vitamin C. Sample 2 was outside the required limit for the first assessment but was within the accepted limit for the second assessment. Therefore, all the samples in this study met the deviation and acceptance values as per the British Pharmacopoeia 2018.

The retention factor (RF) of 0.7 for identification of pure vitamin C showed that all the different brands of vitamin C contained ascorbic acid. A study conducted in India also used the retention factor as a method for the identification of active ingredients [9]. In addition, a 2008 study conducted in Nigeria also showed that pure ascorbic acid or vitamin C brands had an RF value of 0.7 [2].

Friability test for all the samples gave a weight loss of <1% revealing that all the samples complied with the BP (2018) specification and that these brands can withstand abrasion without loss of integrity. This friability result is similar to a study conducted in Bangladesh in which most of the samples passed the standard limit of friability (Not more than 1%) [10].

The disintegration test (non-official method) done on the four brands of non-chewable tablets showed that three of the brands complied with the specification of less than 15 min for disintegration time while one of the brands failed the disintegration test. The disintegration test does not apply to chewable tablets BP 2018 [7]. The ability of the three brands to disintegrate within this time indicates that these tablets can break down in the gastrointestinal tract and release their content into the systemic circulation. This result is similar to a study conducted in Nigeria for determining the disintegration rates in which disintegration time ranged from 0.32 to 24.03 min, and all the disintegration times were within the BP official limit of 30 min [11].

The assay results for ascorbic acid using titrimetric and colourimetric methods for all the samples were comparable. The results showed that the titration value and colourimetric value for the ascorbic acid were consistent, indicating that each technique is appropriate for determining ascorbic acid. This result means that there was little or no interference of the excipient on the assay procedure [12]. Some of the samples were within the official titration specification of 95.0-107.50% BP (2018). One sample had more than the required active ingredient, possibly because of the addition of overages to vitamins by most manufacturers [13]. One brand failed both titrimetric and colourimetric analysis, maybe due to poor storage at the pharmaceutical outlet or poor handling during transportation from manufacturer to the point of sale or use. Research evidence in Bangladesh showed poor pharmaceutical practices in pharmacies of Bangladesh and confirmed that the regulatory authority has a vital role to play in shaping the behaviour of pharmacies [14].

This study has shown a need for functional evaluation of the quality of drugs in the supply chain through robust pre-and post-market surveillance measures to identify substandard and deteriorated drugs. Furthermore, in ascertaining the quality of different brands of medicine, assay for active constituents should be combined with assessment of other pharmaceutical parameters that include storage conditions and the suitability of the facilities handling the products and personnel.

Our study did not determine the storage condition of the different pharmaceutical outlets. However, this study provided information on the quality of vitamin C and their registration status despite this limitation. It also showed the need for wide-scale post-market research to understand the effect of environmental conditions on drugs and their drug deterioration in the supply chain in different parts of the country.

CONCLUSION

Our study confirmed that one of the nine vitamin C products assessed was unregistered. Also, our findings showed that one of the nine registered samples did not contain the required content of Vitamin C. Our results emphasised the need for pharmaceutical wholesalers and retailers to follow the shelf-life storage recommendations of the manufacturer. The study provides good insight into the importance of conducting routine quality evaluation through post-market surveillance and the need to ascertain good pharmaceutical practice, including good storage and identification of potential substandard and counterfeit medications.

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AUTHORS CONTRIBUTIONS

All authors have read and approved the manuscript. ML and AFM developed the concept and proposal of the study. ML, EBSC, MS guided the research methods.

ML and AMF analysed the dataset while ML, EBSC, ATK, MS, MB, JPJK, and WCNJ provided expert reviews. AMF collected data, and ML and AMF prepared the write-up. ML, EBSC, JA, MB and MS finalised the assessment of the study.

CONFLICT OF INTERESTS

The authors declare no competing interests whatsover.

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