QUALITY OF ANTIRETROVIRAL DRUGS, STAVUDINE AND INDINAVIR CAPSULES AVAILABLE IN THE TANZANIAN MARKET

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Summary

Background: The number of antiretroviral drugs (ARVs) available to HIV/AIDS patients in Tanzania is increasing due to a number of intervention programs such as PEPFAR and the Clinton Foundation. These ARVs are imported from a number of countries. However, currently there are no reports on the quality of these medicines imported into Tanzania.

The sale of substandard and counterfeit drugs has been well documented particularly in developing countries. The marketing of counterfeit and substandard antiretroviral drugs has also been widely reported in Africa. It is therefore important to closely monitor the quality of ARVs marketed in Tanzania to ensure that substandard or fake products are uncovered before great harm is done to public health.

Objective: To assess the quality of ARVs marketed in Tanzania.

Methodology: A total of five samples of two generic drugs (stavudine and indinavir) from different manufacturers were randomly collected from various retail pharmacies.

Assessment of package inserts and labels was carried out using the Tanzania Food and Drugs Authority (TFDA) specifications. The capsules were analyzed for the content of the active components using validated in-house methods

Results: All samples of Indinavir and Stavudine investigated conformed to the packaging and labeling specifications. However, all Indinavir samples were found to contain excess amount of active ingredient (112.6% - 118%) compared to the official limit of 95 – 105%. One sample of stavudine capsules failed the dissolution test, releasing only 56% instead of the specified 80% of the active ingredient.

Conclusion: The results of this study emphasize the need for careful monitoring of the quality of drugs to ensure their safety and efficacy.

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Introduction

Antiretroviral dugs are used to slow disease progression in patients infected with HIV (Human immunodeficiency virus), to prolong life and improve the quality of life. Principally it is recommended to use two or three drugs that is, di- or triple therapy[(1)], in order to delay emergence of resistant strains.

Following the emergence of HIV/AIDS in 1980/81 (and for Tanzania in 1983), antiretroviral drugs started to become widely available worldwide as early as 1995.[(2,3)] In Tanzania these were available from several retail pharmacies in Dar es Salaam. The list constituted mainly zidovudine, stavudine, indinavir and lamivudine. By 2004, 56 antiretroviral products from suppliers in UK, Germany, USA, Italy and India were registered in this country [(4).] These products are in different dosage forms and some of them are combination preparations. They include innovator preparations from companies such as Glaxo Smith Kline Research & Development, Hoffman la Roche as well as generic substitutions from Indian Companies such as Cipla.

Medicines enjoying exemption from taxes in Tanzania are largely those included in the Tanzania National Essential Drugs List (TNEDL). By 2003 antiretroviral drugs had not yet been included in the TNEDL for distribution by public and reputable private institutions at a subsidized price. To compound the problem further, HIV/AIDS patients are also

confronted with complications of opportunistic infections and lack of a balanced diet. With the launching of the Bush program (PEPFAR), antiretroviral drugs have become more accessible [5], but still not accessible to all patients who need the drugs.

It is quite conceivable that under the above-mentioned circumstances, conmen anxious for making quick profits will come up with substandard or counterfeit drugs for the already unfortunate patients. Substandard medicines are products whose composition and ingredients do not meet the correct specifications and which are consequently ineffective and often dangerous to the patient [6]. Substandard products may occur as a result of negligence, human error, insufficient human and financial resources or counterfeiting.

Counterfeit medicines are part of the broader phenomenon of substandard pharmaceuticals [6]. The difference is that they are deliberately and fraudulently mislabeled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit medicines may include products with the correct ingredients but fake packaging, with the wrong ingredients, without active ingredients or with insufficient active ingredients.

The sale of substandard and counterfeit drugs has been well documented particularly in developing countries [7,8]. A study conducted in South East Asia revealed that 38% of artesunate products contained no active ingredient and therefore had no therapeutic value [9]. In Peru up to 8% of drugs on the market were estimated to be counterfeit [10].

The marketing of counterfeit and substandard antiretroviral drugs has also been widely reported in Africa. The 3-in-1 antiretroviral drug (Genovir 3D) was counterfeited and sold in Ivory Coast through a Namibian company [11]. The drug was supposed to contain 200 mg of zidovudine, 150 mg of lamivudine and 40 mg of indinavir. However, a laboratory investigation found no trace of any of the three active ingredients listed on the label, instead each capsule contained stavudine in addition to an unidentified substance. In the Democratic Republic of Congo, the antidepressant fluvoxamine and the muscle relaxant cyclobenzaprine, were labeled and sold as Triomune (a combination of the ARVs stavudine, lamivudine and nevirapine) or Duovir (a combination of the zidovudine and lamivudine).[12]

In 2002 Glaxo Smith Kline in the United States discovered suspected bottles containing tablets of the antiretroviral drug abacavir falsely labeled as combivir, which is supposed to be a combination drug consisting of zidovudine and lamivudine.[6] Both medicines are used as part of combination regimens to treat HIV infection and can cause potentially life-threatening hypersensitivity reactions in patients taking other medicines in the combination. Using poor quality drugs may result into worsening of the disease condition, prolonged illness, death, inducement of toxic reaction and wastage of financial resources. Recently a simpler, yet equally effective, method of fraud has emerged that could have serious consequences for patients through expiry date tampering.[13]

For antiretroviral therapy, the emergence of drug faking would have very grave consequences due to potential explosion of resistant viruses. It is therefore imperative that antiretroviral drugs circulating within the Tanzanian market are closely monitored and incidences of substandard or fake drugs are curbed before gross damage is done to the control of the HIV/AIDS epidemic. This study was therefore conducted to set the trend in surveillance of the quality of antiretroviral drugs.

Methodology

Instrumentation:

Quantitative analysis was performed using a UV-Visible Spectrophotometer Model 2041 (UK), while sonication was carried out with a sonicator, Model 3210 (Branson, UK). Dissolution was performed using a basket apparatus, Erweka (DT 70, Germany).

Samples

Samples of Indinavir (400 mg) and Stavudine (40 mg) capsules were purchased from randomly selected retail pharmacies in Dar es Salaam, by convenience sampling. Two types of antiretroviral drugs were tested; which included Indinavir, a protease inhibitor and Stavudine, a nucleoside reverse transcriptase inhibitor. A total of five products (three of indinavir and two of stavudine) from different suppliers were assessed.

Reference standards were obtained from the Tanzania Food and Drugs Authority (TFDA). Since most antiretroviral drugs were new compounds, without analytical methods in official compendia, at the time of this study, the quality surveillance was, therefore based on general tests and assays, depending on manufacturers' in-house methods which had been validated in all aspects by the TFDA. Assays were performed using a UV spectrophotometer at a wavelength of 260 nm.

Z\Sample Collection

A list of all registered antiretroviral drugs in Tanzania was obtained from the TFDA. As antiretroviral drugs are prescription only medicines, collection was achieved through the use of special permission. The samples were bought from pharmacies with good storage conditions (good ventilation and cooling systems) in order to rule out the possibility of purchasing samples that might have deteriorated due to poor storage conditions.

Assessment of conformity to Packaging and labeling requirements

Each sample was assessed for its conformity to packaging and labeling requirements with reference to TFDA guidelines.

Reference standards

Reference standards consisted of Indinavir sulphate powder (99.02 % purity) and Stavudine powder (97% purity).

Identification test

For each product a solution of the sample was prepared and its UV spectrum obtained for comparison with that of the standard solution.

Assay for active ingredient

Indinavir calibration curve

Indinavir sulphate (27.7 mg) working standard was weighed and transferred into a 50ml volumetric flask. It was then dissolved, with the aid of sonication and diluted to volume with acetonitrile and distilled water mixed in a ratio of 1: 1. For preparation of the calibration curve, 2, 4, 6, and 8 ml of stock solution were withdrawn and diluted further to 25ml with the same diluent, so that the final concentrations were 44.32, 88.64, 132.96 and 177.28 mg/L, respectively. The absorbance of standard solution was measured at the wavelength of maximum absorption (260 nm) using acetonitrile and distilled water (1: 1) as the blank.

Stavudine calibration curve

The working standard of Stavudine (19.8 mg) was weighed and transferred into a 100ml volumetric flask. About 50 ml distilled water added to dissolve the standard with the aid of sonication, then made up to volume (100 ml) with the same solvent. A 10 ml aliquot of this solution was transferred into 100 ml volumetric flask and brought to volume using distilled water to make a concentration of 19.8 mg/L. From this solution 5, 10, 15 and 20 ml were withdrawn individually and transferred into separate 25 ml volumetric flasks and diluted to volume using the same solvent. Standard solutions with concentrations of 3.96 mg/L, 7.92 mg/L, 11.88 mg/L and 15.84 mg/L, respectively, were obtained. The absorbance of standard solution was measured at the wavelength of maximum absorption (265 nm) using and distilled water as the blank.

Analysis of samples

Sample preparation

Ten intact capsules from each product were weighed. The capsules were then opened and the powder was weighed. A portion of the powder equivalent to 25mg from each product was dissolved and diluted with solvents as described under calibration curve. The absorbances of sample solutions were determined spectrophotometrically at 260 nm for indinavir sulphate and 265 nm for stavudine. Based on the equations derived from calibration curves of the working standards the content of active ingredient present in a single capsule was determined.

Dissolution test

Indinavir sulphate working standard

Twenty-five mg of Indinavir sulphate standard were transferred into a 50 ml volumetric flask and about 20 ml of dissolution medium (acetonitrile/distilled water 1:1) added to dissolve the powder. The volume was then made up to 50 ml using the dissolution medium. A 2 ml aliquot of this solution was transferred into a 25 ml volumetric flask and the solution was brought to volume using the same solvent. The resulting solution had a concentration of 40mg/L. The standard solution was analyzed spectrophotometrically at 262 nm and absorbance was determined.

Stavudine working standard

A powder of stavudine standard weighing 40 mg was transferred into a 100 ml volumetric flask and about 50ml of dissolution medium (0.01 N HCl) was added to dissolve the powder. The volume was then made up to 100 ml using the dissolution medium. An aliquot of 5 ml from this solution was transferred into a 200 ml volumetric flask and the solution was brought to volume using the same solvent to make a concentration of 10mg/L. The standard solution was analyzed spectrophotometrically at 265 nm and absorbance was determined.

Dissolution testing of samples

The dissolution rate was determined using the USP basket method [14]. Six capsules of each product were placed separately into 6 dry baskets at the beginning of the test. The dissolution vessel was filled with 900 ml of dissolution medium (0.01 N HCL for indinavir sulphate capsule and distilled water for stavudine capsules) and maintained at 37°C ± 0.5° C. The baskets were then lowered into position and the apparatus was operated at a speed of 100 revolutions per minute. At the end of 30 minutes (Stavudine) and 45 minutes (Indinavir sulphate), a 5 ml aliquot of the test sample solution was withdrawn, diluted to 200 ml and analyzed spectrophotometrically. Absorbance was measured at 262 nm and 265 nm for indinavir sulphate and stavudine test samples, respectively.

Results

Table 1 summarizes the packaging and labeling specifications of TFDA. All samples ARV1 to ARV5 were found to conform to these specifications.

Identification Test

All samples showed the same absorption as their corresponding reference standards (Table 2) confirming presence of the active ingredients in the formulation

Table 1: Packaging and labeling requirements of TFDA

S/N	Observation
1	Non-proprietary and Proprietary Name
2	Dosage form of the product
3	Name and strength of active ingredient
4	Name and address of the manufacturer
5	Precautions, storage instruction and shelf life
6	Batch or Lot number
7	Dates of manufacture and expiration
8	Indication and contraindications
9	Dosage regimen and directions for use
10	Packing and pack size
11	Adverse effects, drug interaction, treatment of overdose
12	Manufacturer Country of origin
13	Date of publication of package insert

Table 2: Results of identification test carried out on the samples

Product	Identification	Remarks
ARV1	Positive	Passed
ARV2	Positive	Passed
ARV3	Positive	Passed
ARV4	Positive	Passed
ARV5	Positive	Passed

The standard solutions of indinavir sulphate and stavudine were found to obey the Beer-Lambert Laws at concentrations of 40 to 180 mg/mL and 4 to 16 mg/mL respectively. Plots of absorbance against concentration for both antiretroviral drugs were linear giving regression equations of $y=0.0054x - 0.016$ and $y = 0.0416x + 0.021$ respectively. The content of active ingredients of each product, expressed as a percentage of the stated amount is presented in Table 3.

Table 3: Content of Active ingredients in the samples

Product	Amount found-(mg)	% amount as per label claim	Manufacturer specification	Remarks
ARV1	453.30	113.3	95 -105 %	FAIL
ARV2	450.34	112.6	95 - 105 %	FAIL
ARV3	472.39	118.0	95 - 105 %	FAIL
ARV4	40.00	100.0	90 – 100 %	Pass
ARV5	38.80	97.0	90 – 100 %	Pass

All products containing Indinavir sulphate did not comply with the in-house specifications of active ingredients, by containing much more than the specified amount. Stavudine products complied with the given specifications (See Table 3 and Figure .

Content Determination

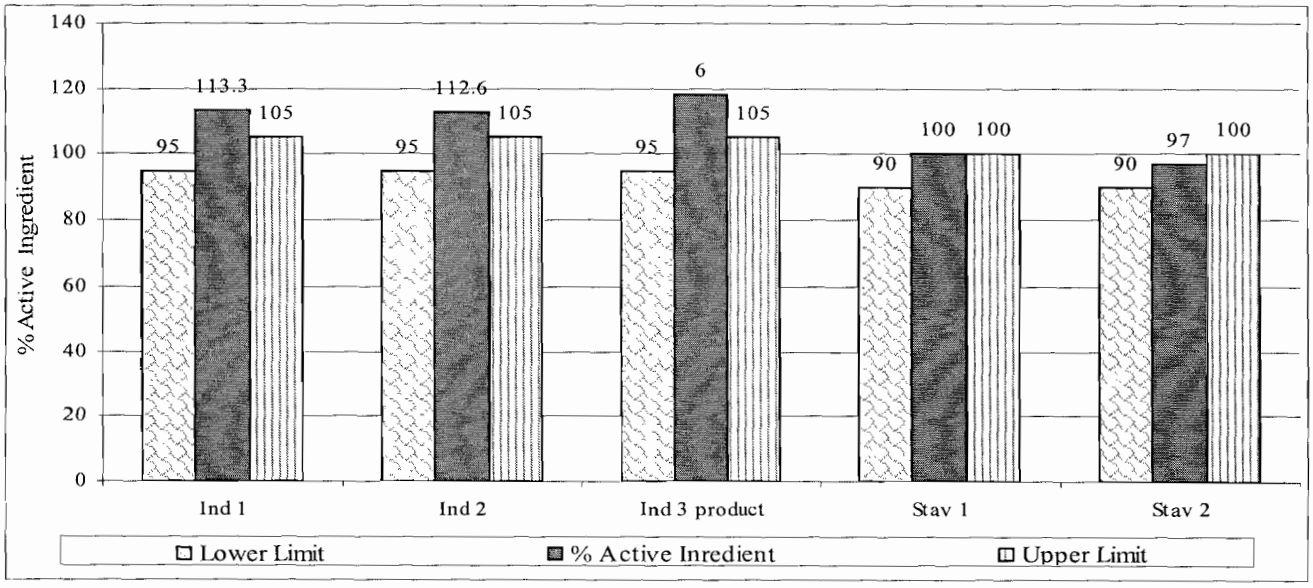

Figure 1. Comparison of percentages of Active Ingredients in the samples with minimum and maximum in-house specifications

Dissolution test

Manufacturers' specifications require that during the dissolution testing the formulation should release not less than 80% of the labeled amount in 30 minutes for stavudine and 45 minutes for indinavir sulphate capsules. Four of the samples (ARV1, ARV2, ARV3 and ARV5) complied with the in-house specifications but one product (ARV4) failed (Table 4).

Table 4. Percentage release of active ingredient of the five samples

Product	% Released	Manufacturer specification (≥)	Comments
ARV1	96.25	80% in 45 minutes	Pass
ARV2	97.15	80% in 45 minutes	Pass
ARV3	97.10	80% in 45 minutes	Pass
ARV4	56.00	80% in 30 minutes	Fail
ARV5	95.79	80% in 30 minutes	Pass

Discussion

The surveillance of quality of ARVs circulating in the Tanzanian market encompassed examination of outer and inner drug package, package insert, identity tests, assay for the content of active ingredient, as well as, dissolution rates, to assess compliance with TFDA requirements and manufacturers' own specifications. All these parameters play an important role in the performance of the product throughout its shelf life.

The outer and inner packages serve to protect tablets and capsules from moisture, light, crushing and mechanical shock.[15] Any evidence of deviation from package specifications obtained from visual inspection will therefore indicate possible exposure of the pharmaceutical product to adverse conditions.[16] In resource limited pharmaceutical supply institutions, the presence of substandard packages and package inserts alone can serve as an important parameter in the selection of reliable suppliers in the overall process of drug quality assurance.[17] All five ARVs selected in this study complied with packaging and package insert requirements.

Identity tests serve to confirm the presence or absence of the active ingredients. The identity of an active ingredient can be determined by chemical tests such as colour reactions involving a functional group in the molecule or a physical test such as interaction with ultraviolet light. In this case a comparison of the ultraviolet spectrum of sample ARVs against authentic standard confirmed that all five samples had the active ingredients as indicated on the labels.

The assay for content of active ingredients was performed using ultraviolet spectrophotometric methods for both stavudine and indinavir. According to manufacturers' in-house specifications, indinavir was supposed to contain 95 to 105% of the active ingredient whereas stavudine 90 to 100%. All products containing Indinavir sulphate did not comply with the in-house specifications of active ingredients, by containing much more than the specified amount. Stavudine products complied with the given specifications. A logical explanation for the excess active ingredient in ARV1, ARV2 and ARV3 was not apparent but it could have something to do with addition of an overage for indinavir. Some manufacturers add excess active ingredient to guarantee the potency of their product throughout its shelf life. However, the addition of any overage to a registered product must be stated and justified.[18]

For the dissolution rate out of the five samples 4 exhibited satisfactory release characteristics but one stavudine sample released only 56% within 30 minutes. Dissolution testing is an important tool for predicting bioavailability, and in some cases, can replace clinical studies to determine bioequivalence[19]. Failing a dissolution test is certainly good evidence of a poor quality drug. The samples were collected from pharmacies with good storage conditions. Therefore the poor quality of this sample could be associated with poor manufacturing processes resulting in slow rate of de-aggregation such that the capsule mass still maintained its capsule like shape after 30 minutes. It could also be attributed to bureaucracy during clearance of consignments at ports of entry, which could lead to drug spoilage due to+storage under inappropriate conditions.

Taking into account that anti-retroviral drugs are very expensive, it is obvious that the generic substitution products could be the most cost effective treatment for patients in the least developed countries. In 2002 it was estimated that patented drugs cost around $10,000 to $15,000 per patient per year while generic substitutes cost around $1,500 to $2,000 per patient per year.[20] Thus, the majority of the people cannot afford to buy expensive branded drugs, as the national per capita income of Tanzania in 2004 was US $ 300.[21]

However it important is to ensure that they exhibit the same therapeutic effectiveness as the brand products. This study has revealed the presence of products circulating on the Tanzanian market that do not comply with the required specifications. One product displayed substandard drug release and three products contained excessive amount of active ingredient. This emphasizes the need to be more vigilant in post-marketing drug surveillance.

Conclusion

This study has revealed that ARVs marketed in Tanzania are not strictly according to specification. In other words they are sub-standard. This poses a potential danger to HIV/AIDS patients who depend on these products to prolong and improve the quality of their lives. If manufacturers can get away with products that are not strictly according to specifications, they can as well circulate substandard and counterfeit products. It is therefore stressed that pharmacovigilance monitoring be regularly carried out by all relevant stakeholders.

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