ORIGINAL PAPER

Nig. J. Pharm. Res. 2017, 13 (2) pp 97-104
ISSN 0189-8434 **Available online at http://www.nigjpharmres.com**

Comparative *In Vitro* Quality Assessment of Five Brands of Furosemide Tablets Marketed in Port Harcourt, Nigeria

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Medication failure, high morbidity and mortality resulting from the circulation and consumption of fake, adulterated and substandard medicines have been a worrisome issue to health practitioners, patients and drug regulatory agencies of Nigeria.

Objective: This study aims at evaluating some brands of furosemide tablets that are marketed in Port Harcourt, Nigeria to know if they meet with their label claims and British Pharmacopoeia (BP) or the United States Pharmacopoeia (USP) set limits for such products.

Materials and Methods: Five brands of furosemide tablets marketed in Nigeria were randomly collected from different retail pharmacy outlets and investigated for uniformity of tablet weight, disintegration, friability, hardness, and drug release profile using standard methods. Two assay methods based on titrimetry and spectrophotometry were employed for the determination of the content of furosemide in the tablet formulations or its bulk drug.

Results: Results obtained showed tablet weight in the range of 174 mg ± 0.05% to 274 mg ± 0.01 %, hardness ranging from 3.20 ± 0.01 to 10.70 ± 1.70 kg/F, friability of < 1 %, disintegration time of 5.20 ± 0.88 to 9.30 ± 0.50 min, drug release of > 80 % within 30 min and assay of 86.45 to 100.80 % .

Conclusion: Most of the tablet batches tested met with label claim in terms of the content of furosemide and also complied with acceptance limits of the British Pharmacopoeia (BP) or the United States Pharmacopoeia (USP) and were adjudged to be of good quality.

Keywords: Furosemide, active pharmaceutical ingredient, British Pharmacopoeia, United States Pharmacopoeia.

INTRODUCTION

Many countries of the world especially the third world countries are faced with the menace of substandard, fake or adulterated drug, treatment failure, and drug toxicity amongst other undesirable adverse health implications arising from the circulation of unwholesome drug products. The World Health Organisation, WHO estimates that about 10 % of the world's pharmaceutical trade (25 %) in developing countries consists of fakes or substandard products (Pincock, 2003, Gibson, 2004), while up to 25 % of all drugs consumed in poor countries are alleged to be counterfeit or substandard (Rudolf and Bernstein, 2004). According to the WHO, counterfeit medicines are medicines that are deliberately and fraudulently mislabelled with respect to their identity and/or source. This definition is applicable to both branded and generic products. Medicines regarded as counterfeit may include medicines with correct ingredients, wrong ingredients, without active ingredients, with incorrect amounts of active ingredients or with fake packaging (WHO, 2010). Substandard medicines are defined as products whose composition and ingredients do not meet the correct

scientific specifications and are consequently ineffective and often dangerous to the patient. The use of counterfeit medical products is a global public health problem which may result in death, disability and injury to the consumer. Illegitimate distribution and rampant use of counterfeit medicines could cause loss of confidence in health systems and healthcare providers (WHO, 2003).

Furosemide is an essential drug that is applied in life-threatening situations and in most cases to a special population-geriatrics. This tender group of the population/patients need to receive the accurate doses of medication that would elicit good therapeutic responses with minimal untoward health implications. Furosemide chemically known as 5-(aminosulfonyl)-4-chloro-2-[(2-furanylmethyl) amino] benzoic acid, is structurally a sulfonamide, an antibacterial agent and a potent diuretic. It is most commonly used as a diuretic in the treatment of edematous conditions associated with chronic renal failure (Rahway, 2007, Patel and Solanki, 2012), hypertension, congestive heart failure (Foye *et al*, 2008), and cirrhosis of the liver (Gringauz, 2007). Diuretic agents are drugs that increase renal excretion of water and solutes especially sodium salt (Das and Senapati, 2007)). The target primary organ of diuretics is the kidney where it exerts a predominant activity in the Henle's loop producing a remarkable diuresis (Delgado and Remers, 1991). It inhibits the uptake of sodium and chloride in the proximal and distal tubules as well as the Henle's Loop. Furosemide is acidic and has a pKa of 3.9. It is commercially available in dosage strengths of 20, 40 and 80 mg tablets for oral administration and 40 mg/mL for parenteral administration. It is practically insoluble in water, poorly soluble in alcohol and freely soluble in dilute alkali solutions (Dos-Santos *et al*., 2011). Furosemide is highly bound to plasma proteins especially albumin with about 91 – 99 % of it being bound in healthy individuals. The onset of action following oral administration is within 1 h, with peak values being reached within 2 h, while the duration of action or effect is about 6 – 8 h. Furosemide has a terminal life of approximately 2 h (Edwin, 2006).

Studies reveal that several methods have been employed in the analysis of furosemide tablets amongst which is titrimetry and spectrophotometry. Several spectrophotometric methods have also been reported for the determination of furosemide in bulk, pharmaceutical dosage forms, and/or biological fluids (Gahandule and Banerjee, 2016).

Tharpa *et al*., (2009), described two visible spectrophotometric methods which were based on the reduction of potassium permanganate ($KMnO_4$) in acidic and basic media. In method A, furosemide was treated with a measured excess of permanganate in acidic medium and the unreacted oxidant was measured at 550 nm, whereas in method B the reaction was carried out in alkaline medium and the resulting manganate was measured at 610 nm (Tharpa, *et al*., 2009) The choice of a method to use would depend on factors such as the simplicity, sensitivity, wide linear ranges, mild experimental conditions and above all the cost- effectiveness of the proposed methods.

Materials

Sodium hydroxide (Qualikems Laboratory Reagents, India), oxalic acid (Hangzhou Dayangchem Co., Ltd., China), phenolphthalein (Aldrich Chem Co, USA), bromothymol blue, five brands of furosemide tablets, distilled water, reference sample furosemide (Sigma-Aldrich, USA).

Method

Five brands of furosemide were purchased from different pharmacy retail outlets in Port Harcourt, Rivers State. Each brand was physically inspected and found to be properly strip packed and intact with the appropriate product information: brand name, strength, pack size, country of manufacture, manufacturing and expiry dates, National Agency for Food and Drug Administration (NAFDAC) registration number clearly stated. The five brands were labeled Furo A, Furo B, Furo C, Furo D and Furo E. Only one brand amongst the five was manufactured in Nigeria, the remaining four were manufactured in India. One hundred (100) tablets were randomly selected from each brand/batch and used for the following tests:

Uniformity of weight test

From each batch, twenty tablets were randomly removed from the blisters in which they were strip packed and used for the test. The tablets were weighed singly and later collectively using a digital analytical balance (Adventurer®, England). The mean weight, standard deviation and coefficient of variance were determined.

Hardness test

Ten tablets randomly selected from different strip packed batches of the furosemide tablets were tested for their hardness/crushing strength using a Mosanto® tablet hardness tester (Monsanto, India). The mean crushing strength, standard deviation and coefficient of variance were determined for each batch.

Friability test

Twenty tablets were randomly selected from each batch and used for the test. They were dedusted by

directing a stream of air unto them, weighed and placed in an Erweka twin drum friabilator (Erweka®, Germany) set to rotate at 25 revolutions per minute (rpm) for 4 minutes after which the tablets were dedusted and reweighed. The friability, F was calculated as the percentage of the difference in weight of the tablets before(W_o) and after (W_f) the test as expressed in Equation 1(Ofoefule, 2004). Replicate determinations were done for each batch.

$F = [W_o - W_f/W_o] \times 100\ \%$ ……1

Disintegration test

Six tablets randomly selected from each batch of the furosemide tablets were used for the test. Each tablet was put into the cylindrical hole of each of the six holes of the basket assembly of the disintegration tester (Erweka® ZT-3 double basket, Germany) and each tablet held in place with a glass disc. Each beaker was filled with 500 mL of 0.1 N HCl heated up to 37 ± 1˚C. The time taken for each tablet to completely break up and completely pass through the mesh was noted. Triplicate determinations were done. The mean and standard deviation of each reading was determined. The same procedure was used for the different batches of the furosemide tablets.

Assay of drug/drug content

(a) Titrimetry

(i) Preparation and standardization of 0.1 M sodium hydroxide solution

A preparation of 0.1 M sodium hydroxide (0.1 M NaOH) was made. Also, 0.1433 g of dry oxalic acid was dissolved in 50 mL of distilled water. Using three (3) drops of phenolphthalein as indicator, the oxalic acid solution was titrated against the 0.1 M NaOH until a pink colour was obtained. The procedure was run in triplicates and the average titer determined. The equation of the reaction between oxalic acid and NaOH and its milliequivalent relationship is:

$$H_2C_2O_4 + 2NaOH \longrightarrow NaC_2O_4 + 2H_2O$$

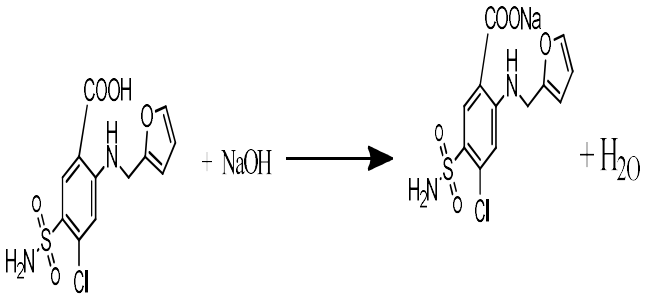
$$0.00450\text{mg of } H_2C_2O_4 = 1\text{ml } 0.1\text{M NaOH}$$

(ii) Assay

Twenty tablets of furosemide were randomly collected from each batch. The tablets of each batch were collectively weighed and pulverized in a mortar. An amount equivalent to one tablet (the mean weight) was taken and dissolved in a solution of 20 mL dimethylformamide. This was titrated against a 0.1 M NaOH solution using 0.2 mL bromothymol blue solution as indicator.

A blank titration was also conducted. The amount of furosemide in each sample was calculated using the milliequivalent relationship between furosemide and NaOH.

$$33.07\text{mg of } C_{21}H_{11}ClN_2O_5S = 1\text{ml } 0.1\text{M NaOH}$$



(b) Spectrophotometry

(i) Determination of wavelength of maximum absorption (λ_{max}) and standard calibration curve.

Ultra violet (UV) spectrometry was employed for the determination of the maximum wavelength of absorption using a JENWAY 6405 spectrophotometer. A 100 mg of a pure furosemide powder was weighed and dissolved in a 100 mL volumetric flask using 0.1M NaOH solution. The volume was made up to 100 mL using 0.1M NaOH to obtain a 1 mg/mL preparation which was scanned in the spectrophotometer to obtain the wavelength of maximum absorption (λ_{max}) at 271 nm**.** Dilutions to obtain 2, 4, 6, 8 and 10 mg % of the preparation were made and each of the diluted samples scanned in the spectrophotometer at 271 nm. The concentrations of the different dilutions were obtained from the corresponding absorbance readings. These values were used to plot the standard calibration curve of the pure sample of furosemide.

(ii) Assay of furosemide tablets

Twenty tablets were randomly selected from batch A of the furosemide tablets and weighed collectively. They were powdered together in a mortar and a quantity equivalent to 250 mg of furosemide powder taken. This was shaken with 300 mL of 0.1 M NaOH to extract the furosemide. The extract was made up to 500 mL with the same solvent. The extract obtained was filtered using a Whatman No 4 filter paper. A 5 mL volume of the extract was collected and diluted up to 250 mL using 0.1 M NaOH solution. The absorbance of the filtrate was determined using the UV/VIS JENWAY 6405 spectrophotometer at 271 nm. The procedure was repeated using batches Furo B, Furo C, Furo D and Furo E of the furosemide tablets.

Dissolution test

The dissolution profile of the different brands of furosemide was investigated for the release of their active pharmaceutical ingredient. A six station dissolution equipment model DT 600 (Erweka®, Germany) was used. Each flask was filled with 900 mL of phosphate buffer (pH 5.8) which was heated to 37 ± 1°C and the paddle speed set at 50 rpm. One tablet from each batch was used introduced separately into the different flasks and 5 mL samples withdrawn at determined time intervals. Five (5) mL replacements of withdrawn samples were made with dissolution media maintained at 37 ± 1°C after each withdrawal. Filtrates of withdrawn samples were assayed for their content using a JENWAY 6405 spectrophotometer set at 271 nm and absorbance readings converted to concentrations using the calibration curve earlier determined.

Statistical analysis: Data were statistically evaluated using Microsoft excel 2010 and Graphpad prism 5.01(GraphPad Software Inc., San Diego, USA)

RESULTS AND DISCUSSION

Physical assessment of tablets

Table 1 shows the results of the physical assessment of the products. Each product was found to have been properly strip packed, with the names of the API, strength and expiry dates written. The packets containing the strip packs also had the product name, strength, pack size, batch number, NAFDAC registration, date of manufacture and expiry, and Manufacturer's address boldly written. All products were intact and not tampered with.

Table 1: Some relevant information on the package of five brands of furosemide tablets

Sample Code/batch	Country of manufacture	Date of manufacture	Expiry date	NAFDAC* status	Tablet strength
FURO A	India	01/2016	12/2018	Registered	40 mg
FURO B	India	04/2015	04/2018	Registered	40 mg
FURO C	India	07/2015	07/2018	Registered	40 mg
FURO D	Nigeria	07/2015	01/2019	Registered	40 mg
FURO E	India	05/2015	04/2018	Registered	40 mg

*NAFDAC= National Agency for Food and Drug Administration

Uniformity of weight

Weight variation or uniformity of the furosemide tablets are shown in Table 2. It was observed that tablet batches Furo A, Furo B, Furo C, Furo D and Furo E had coefficient of variance values of < 5 %. Thus tablets from all the batches tested can be adjudged to have passed the uniformity of weight test for uncoated tablets as stipulated by the British Pharmacopoeia (BP, 2012). This implies that there would be minimal variation or a fair distribution of the active pharmaceutical ingredient and excipients in each tablet manufactured.

Table 2: Some physical parameters of furosemide tablets

Batch	Weight [CV(mg %)]*	Hardness (kg/F)	Friability (%)	Disintegration (min)
FURO A	183.00 (0.01)	7.00 ± 0.82	0.06 ± 0.01	8.03 ± 0.41
FURO B	176.00 (0.03)	4.20 ± 0.55	0.17 ± 0.01	6.50 ± 0.15
FURO C	175.00 (0.01)	3.20 ± 0.01	0.05 ± 0.11	5.20 ± 0.88
FURO D	274.00 (0.01)	10.70 ± 1.70	0.11 ± 0.02	9.30 ± 0.50
FURO E	174.00 (0.03)	3.60 ± 0.70	0.46 ± 0.10	5.18 ± 0.20

*Coefficient of variance of tablets

Hardness

Hardness values for uncoated tablets (Table 2) are expected to be ≥ 4 kg/F in order for such tablets to be able to withstand the shocks of handling and transportation which the tablets may undergo (BP, 2012). Tablet batches Furo C and Furo E fell short of this value (3.00 ± 0.01 and 3.60 ± 0.70) and can be assessed not to have passed while batches Furo A, Furo B and Furo D (4.20 ± 0.55 to 10.70 ± 1.70) met with the acceptance criteria and can be said to have passed the hardness test for uncoated tablets. Thus the tablets that passed are considered to be strong enough to withstand the stresses/rigours of handling and transportation that may be encountered in the manufacturing plant such as coating, printing and packaging or in the drug distribution chain or in the field by the end users/consumers while those that failed the test may crumble or chip under similar stresses or conditions (USP 2009).

Friability

The friability values of the furosemide tablets are shown in Table 2. Values obtained ranged from 0.06 ± 0.01 to 0.46 ± 0.10. The BP requires uncoated tablets to have friability values of ≤ 1 % in order to be able to withstand the abrasion forces they may encounter during handling and transportation (BP, 2012). Furo E tablets were the most friable while Furo C was the least. However, all the batches met with the BP specification (≤ 1 %) and therefore passed the friability test. This implies that the tablets will be physically wholesome despite the various abrasional stresses that could be encountered during handling. Such stresses include those of strip packing, dispensing from bulk packs or other factors that could cause the tablets to rub against each other.Thus there will no loss in the elegance or general appearance of the tablet or its integrity in terms of the content of the active pharmaceutical ingredient.

Disintegration

The disintegration times of the tablets are shown in Table 2. This is the time it takes for a given tablet to break up completely and pass through the mesh of the basket of the disintegration equipment mesh. Values obtained for all the batches were below 15 min which is the British Pharmacopoeial set limit for uncoated tablets (BP, 2012) which implies that all the batches passed the disintegration test. Thus the active pharmaceutical ingredient would be available for dissolution within 15 min of ingestion. This expectedly would aid absorption of the drug after oral administration.

Assay results

Assay results showing the content of active pharmaceutical ingredient using titrimetry and spectrophotometric methods are shown in Figures 1 a & b respectively. All the tablet batches except Furo B complied with the United States Pharmacopoeia set limit for furosemide which states that such tablets should contain not less than 90 % or more than 110 % of the label claim (USP 32, 2009). The similarity of the results obtained by both methods shows that there was little or no technical error by the personnel during the tests and that either method was good and reliable for such evaluation.

Dissolution profile

The dissolution profile of the different batches of furosemide is shown in Fig. 2. There was a sharp release of the active pharmaceutical ingredient within 10 min after which release was sustained but gradual. All the batches released more than 80 % of their drug content within 30 min. Batches Furo A, E and C exhibited a slightly greater release than Furo D and B. All the batches released more than 80 % of their drug content within 60 min and can be adjudged as compliant with the USP specification for furosemide tablets. The USP stipulates that not less than 80 % of furosemide be released from the tablets within 60 min (USP, 2009). Both the imported brands and the made in Nigeria or locally manufactured brand were maximally released within 60 min. Thus all the batches passed the dissolution test and their active pharmaceutical ingredient would be relatively bioavailable for absorption when ingested.

CONCLUSION

The physical evaluation results of the different batches showed that the tablets were wholesome and had not been tampered with which implies that the products were nor faked. Other tablet evaluation parameters such as uniformity of tablet weight, disintegration, hardness and friability were within British Pharmacopoeial acceptable limits except for Furo B and D whose hardness values were below acceptable limits of ≥ 4 kg/F. Both titrimetric and spectrophotometric methods of furosemide assay showed good results that were comparable. The content of active ingredient did not differ from label claim for all the batches and these were found to comply with the BP and USP standards. The dissolution profile showed all the batches releasing up to 80 % of their active pharmaceutical ingredient within 30 min which complied with the USP set limit. The locally manufactured brand (Furo D) compared well with imported brands Furo A and B in

all the parameters assessed while Furo C and E fell short of its standard only in terms of hardness. Since the other assessment parameters for the mechanical strength, disintegration, content of active ingredient and dissolution were met with by all the batches/brands, it can be concluded that the locally manufactured brand and the imported brands of furosemide marketed in Port Harcourt, Nigeria are comparable and meet with NAFDAC and pharmacopoiel requirements in terms of physical assessment parameters for uncoated tablets, label claim, dissolution and are therefore not fake, adulterated or sub-standard but fit for consumption.

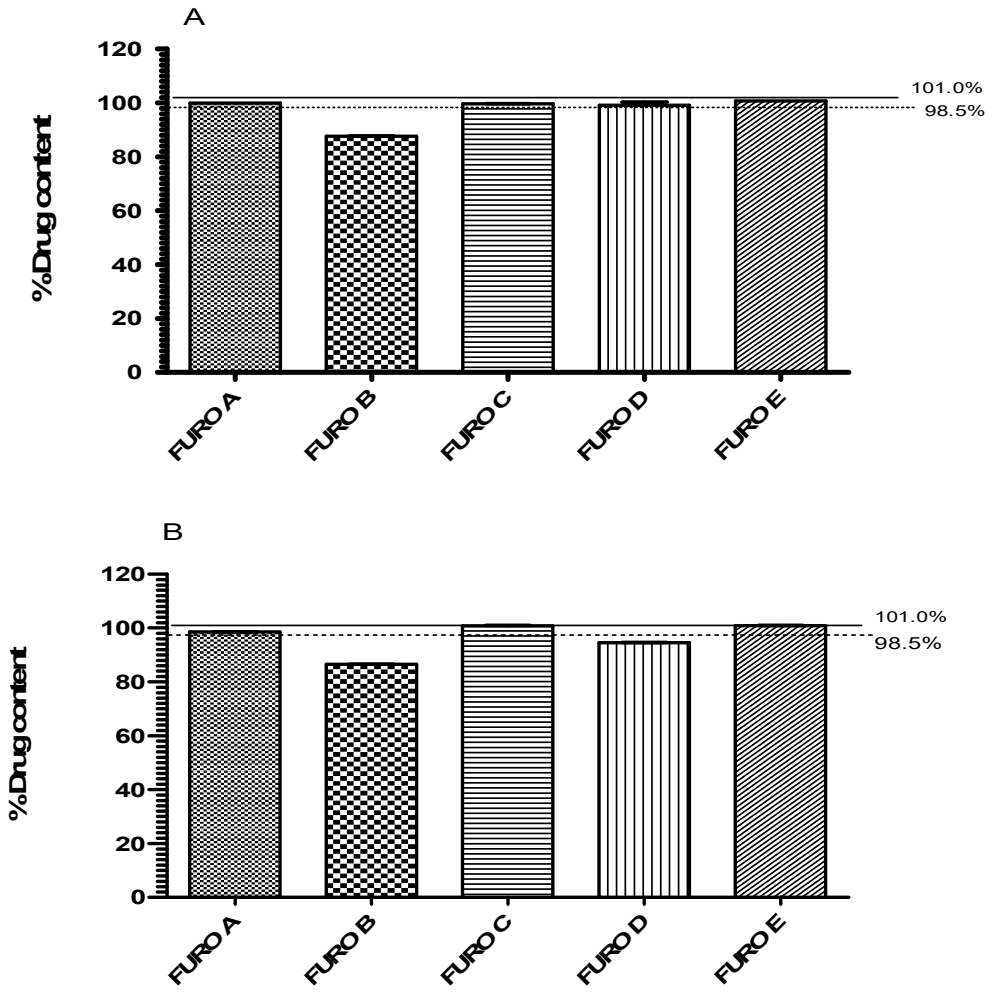


Fig. 1(a&b): Assay plots of furosemide tablets [A shows titrimetry method while B shows spectroscopy method].

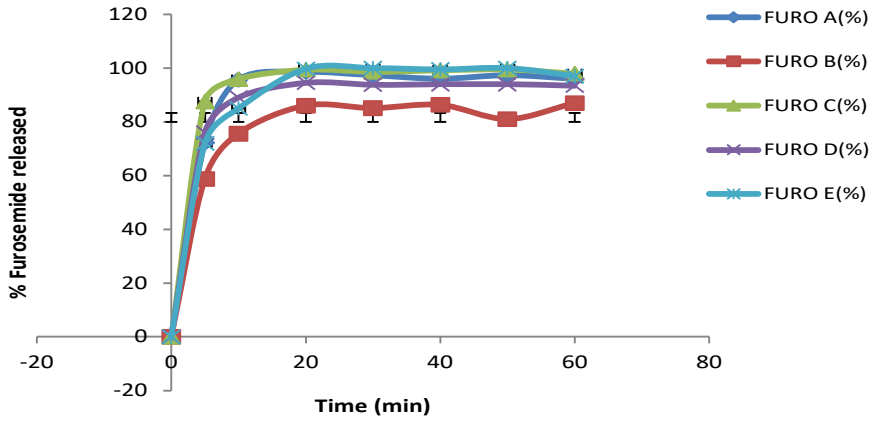


Fig. 2: Percentage furosemide released against time

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Conflict of Interest: None declared

Received: 16 August, 2017

Accepted: 13 December, 2017