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Qualitative and Quantitative Evaluation of Multi-source Piroxicam Capsules Available in Nigeria.

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Abstract
The qualitative and quantitative evaluation of eleven brands of piroxicam capsules marketed in Nigeria is presented. The disintegration time, dissolution rate and absolute drug content were determined in simulated intestinal fluid (SIF) and simulated gastric fluid (SGF) without enzymes. Weight uniformity test was also performed according to official methods. Data obtained from the dissolution profiles in both media (SIF and SGF) were mathematically treated according to the model proposed by Khan (with slight modification) and the resulting predicted availability equivalent (PAE) was used to quantitatively assess/ predict bioavailability of piroxicam from the various brands. Our results indicate variable PAE with pH. In SGF, four out of the eleven (11) brands could be adjudged to be bioequivalent with the innovator drug, Feldene (Neimeth, Nigeria); In SIF, only three out of the eleven (11) were equivalent with the innovator drug. All the capsules (except one brand) generally disintegrated within 15 min. in both SIF and SGF. The weights were also very uniform with insignificant variations. However, the variation in absolute drug contents were generally wide, with all the capsules having drug contents that are above the label claim

Key Words: Piroxicam, Qualitative, Quantitative Evaluation

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Introduction

The influx of multisource (generic) drug products of various classes into the Nigerian market is increasing daily at an alarming rate. This calls for concern especially as it has been observed that the quality of medicines available in some less developed countries (including Nigeria) are poor in terms of level content of active ingredients (Taylor *et al*, 2001; Shakour, *et al*, 1997; Kibwage *et al*, 1992).

Multisource piroxicam capsules abound in the Nigerian open market. Diverse brands from India, China, Malaysia, Belgium, Germany and others are actively competing with the innovator brand (Feldene^R, Pfizer) in terms of price. These brands are relatively cheaper than Feldene^R and as such economically appeal more to the generality of consumers. Even the innovator brand has about two variations with strikingly different prices. A current health concern therefore should be to ascertain the quality (in terms of content of active ingredients) and the potential therapeutic benefit in terms of bioavailability of these multisource piroxicam capsules marketed in Nigeria.

Multisource drug products must satisfy the standards of quality, efficacy and safety as those applicable to the innovator's product (Feldene^R). It is usually not enough to ascertain just the quantity of active constituent (label claim) of the product. This is because biopharmaceutical studies have shown that bioavailability, and hence therapeutic efficacy of most drugs are significantly affected by formulation factors such as binder type or concentration and/ or method of incorporating (intra- and or extra granularly), lubricant type and concentration, particle size of active components, etc (Babalola et al, 2001a; 2001b; Proudfoot, 1988; Jones et al, 1988). Since these factors vary significantly from one manufacturing firm to another, the possibility of differential bioavailability of drugs from similar dosage forms produced by different companies is very high. Using either *in vitro* or *in vivo* designs, several workers in Nigeria have demonstrated differential (variable) release and bioavailability of various drugs from multi-source (generic) dosage forms (Adikwu *et al*, 2001; Ibitayo *et al*, 2001;Ilupeju *et al*, 2001; Ofoefule *et al*, 2001; Ofoefule *et al*, 2000).

Ideally, bioavailability studies should be carried out *in vivo*. However, economic and ethical reasons demand alternative *in vitro* methods since in many developing countries (like Nigeria), in vivo bioavailability/ bioequivalence testing remains an impracticably costly proposition (Olaniyi, 2001). Therefore, *in vitro* drug availability methods that could predict *in vivo* availability of suitable drugs are becoming very popular. One of such tests, based on the conventional dissolution studies, has been successfully used by Ofoefule *et al* to predict the *in vivo* bioavailability of some commercially available brands of perfloxacin and ciprofloxacin marketed in Nigeria (Ofoefule *et al*, 2001; Ofoefule *et al*, 2000).

The current study attempts to investigate the quality of 11 brands of piroxicam marketed in Nigeria *vis-à-vis* pharmacopoeial requirements for content uniformity, weight uniformity, disintegration time and by estimating their possible *in vivo* availability using a modification of the concept of dissolution efficiency proposed by Khan (1975).

Materials and Methods

Materials:

Drug samples-:

Reference sample of piroxicam powder was kindly supplied by Pfizer Nigeria, Plc (now Neimeth Nig, Plc).

Eleven brands of piroxicam capsules (Pixicam^R, Saldin^R, Sanwin^R, Diovin^R, Felvin^R, Felxicam^R, Neoxicam^R, MF-20^R, Piroxy^R, Feldene$_1$^{R} and Feldene$_2$) were purchased from pharmacy shops in Nsukka, Enugu State, Nigeria. Feldene$_2$ is another market variation of the innovator brand suspected to be an imitation of the original Pfizer product, Feldene$_1$.

Chemicals:

Concentrated hydrochloric acid (BDH), and methanol (Mallinckrodt) were purchased locally from chemical stores in Nsukka.

Methods:

Preparation of dissolution Media:

Simulated gastric fluid (SGF) without enzymes was prepared by adding 42.4 ml of concentrated HCl (36.5%) to 2.0 litres of portable water; 10g of NaCl was added to this solution and mixed until complete dissolution occurs. The solution was then made up to 5.0 litres with portable water.

Simulated intestinal fluid (SIF), without enzymes was prepared by dissolving 40g of sodium hydroxide and 34 of monobasic potassium phosphate in 2.0 litres of portable water. The solution was made up to 5 litres with portable water.

Weight Uniformity Test:

Ten capsules were randomly selected from each brand. The intact capsules were opened one after the other and the entire content weighed, making sure that no powder is lost. The mean weight of the capsules and standard deviations in weight was computed.

Disintegration Time Test.

The BP (2001) method was adopted using the Erweka disintegration apparatus. Simulated gastric fluid (SGF) or simulated intestinal fluid (SIF) was used as disintegration medium. The temperature of the apparatus was maintained at $37^0C \pm 1^0C$). One capsule from each brand was placed in 500 ml of either SIF or SGF and the motor switched on. The time taken for the capsule to disintegrate, such that no residue (except fragments of the capsule) remained on the screen, was monitored with a stopwatch. The experiment was repeated twice and the mean time taken.

Dissolution Rate of Capsules

The BP (2001) method was similarly adopted using the Erweka Dissolution Unit with in- built paddle and thermostated electric water that was set to regulate the temperature of the dissolution medium at $37^0C \pm 1^0C$.

A 500 ml quantity of freshly prepared dissolution medium (SGF or SIF) was equilibrated to a temperature of 37 ± 1^0C. From each brand, one capsule was randomly selected and put into the

dissolution medium. At various time intervals ranging between 5-120 min, 1ml of solution was withdrawn from dissolution media and replaced with equivalent quantity of fresh media. The withdrawn sample was filtered, and the filtrate diluted with 9 ml of methanolic HCl (made from 0.77 ml of conc. HCl to 250 ml of methanol to form 0.1N solution HCl in methanol). The absorbance of the resulting solution was measured at 242 nm, against a blank methanolic hydrochloride solution. The concentration of piroxicam was estimated mathematically from the Beer-Lambert's plot.

Beer- Lambert's plot:

Varying concentrations of the reference sample of piroxicam ranging from 0.0031- 0.1 mg% in methanolic hydrochloride were prepared and their absorbances at 242nm determined.

A graph of absorbance versus concentration of piroxicam was plotted and represented as standard Beer-Lambert's plot for piroxicam in methanolic hydrochloride.

Analyses of Data

A modification of the concept of dissolution efficiency was employed to analyze the dissolution rate data. Conventionally, dissolution efficiency, DE is given by,

$$DE = \frac{\text{AUC at time x}}{\text{AUC over the entire time course of release}}$$

where DE = dissolution efficiency

AUC = Area under the release (dissolution) time curve.

However, this concept has been slightly modified and Predicted availability equivalent(PAE) is more appropriately used and is given by,

$$DE^{X} = \frac{\text{AUC of brand X}}{\text{AUC over the entire time curve}}$$

$$DE^{I.B} = \frac{\text{AUC of innovator brand}}{\text{AUC over the entire time curve}}$$

$$PAE = \frac{DE^{X}}{DE^{I.B}} = \frac{\text{AUC of X}}{\text{AUC of innovator brand}}$$

The AUC is estimated mathematically using either Simpson s or trapezoid rule. Differences in PAE of the various brands were ascertained by subjecting the data to statistical analysis (student' t test at 5% level of significance

Results and Discussion

All the brands had fairly uniform weights, with % standard deviation generally very much less than 10%. According to the Europian Pharmacopoeia (2000) specification, capsules with weight deviation less than 10 % have passed the weight uniformity test.

Table 1:Weight uniformity of multi-sourced Piroxicam capsules marketed in Nigeria

Brand	Saldin20	Pixicam20	Felvin20	Felxicam	Feldene2	Diovin20	Piroxy	Sanwin	Neoxicam	Mf20	Feldene
Capsule No											
1	0.3533	0.2849	0.2347	0.3457	0.2644	0.2608	0.3709	0.2845	0.3118	0.2068	0.3208
2	0.3565	0,2930	0.2359	0.3500	0.2641	0.3050	0.3579	0.3000	0.3069	0.2189	0.3215
3	0.3552	0.3225	0.2420	0.3448	0.2584	0.2459	0.3573	0.2833	0.3128	0.2300	0.3312
4	0.3553	0.3213	0.2508	0.3475	0.2632	0.2449	0.3618	0.1627	0.3270	0.2017	0.3225
5	0.3664	0.3112	0.2468	0.3475	0.2512	0.2633	0.3626	0.2826	0.3225	0.2215	0.3153
6	0.3518	0.2813	0.2430	0.3470	0.2675	0.2456	0.3492	0.2889	0.3005	0.2192	0.3221
7	0.3918	0.3141	0.2490	0.3438	0.2616	0.2510	0.3538	0.2568	0.3115	0.2050	0.3220
8	0.3508	0.2656	0.2433	0.3537	0.2504	0.2573	0.3561	0.2499	0.2978	0.2169	0.3241
9	0.3509	0.3112	0.2440	0.3457	0.2526	0.2534	0.3638	0.2785	0.3099	0.2011	0.3318
10	0.3547	0.2900	0.2368	0.3493	0.2565	0.2508	0.3546	0.2936	0.3106	0.2099	0.3250
Average Weight	0.35867	0.2995	0.2426	0.3475	0.2587	0.2578	0,3588	0.2781	0.3111	0.2131	0.3236
Mean ±SD	0.359± 0.01	0.300± 0.02	0.243± 0.005	0.348± 0.003	0.259± 0.006	0.258± 0.02	0,359± 0.006	0.278± 0.02	0.311± 0.009	0.213± 0.01	0.323± 0.005
% Deviation	2.75	6.66	0.41	0.86	2.3	7.75	1.67	7.19	2.89	4.69	1.24

Table 2: Disintegration time of capsules in SIF and SGF

BRANDS	TIME (mins)/ FLUIDS	
	SGF	SIF
SALDIN20	3	6
PIXICAM20	4	8
FELVIN20	10	13
FELXICAM	4	10
FELDENE2	18	25
DIOVIN20	4	12
PIROXY	4.5	6
SANWIN	6	13
NEOXICAM	8	16
MF20	7	8
$FELDENE_1$	6	7

Table 3: Predicted Availability Equivalence (PAE) of Multi-sourced Piroxicam Capsules Marketed in Nigeria.

BRAND	Predicted Availability Equivalence (%)	
	SGF	SIF
SALDIN20	102.0	108.2
PIXICAM20	53.2	94.6
FELVIN20	137.1	80.3
FELXICAM	143.9	128.3
$FELDENE_2$	84.0	44.7
DIOVIN20	104.4	79.0
PIROXY	88.2	78.3
SANWIN	95.8	83.0
NEOXICAM	89.0	94.3
MF20	132.4	86.3
$FELDENE^R_1$	100	100

The disintegration time of capsules in SIF and SGF is shown in table 2. Officially, they are require to disintegrate within 30 min (B.P., 2001). All the capsules therefore passed the disintegration test i both media. However, less time (faster disintegration) was observed in SGF was observed in SGF

The area under the dissolution –time curve was computed mathematically from dissolutio profile (release rates) of piroxicam from the various brands of piroxicam in SGF and SIF and use to estimate the predicted availability Equivalence (PAE) according to the relationship:

$$PAE = \frac{\text{AUC of drug } \chi \text{ X } 100\%}{\text{AUC of Feldene}^{R} \text{ (innovator brand)}}$$

The PAE values of the various brands in SIF and SGF are presented in table 3.
PAE values were generally higher in SGF than in SIF. The PAE of Saldin 20, Diovin 20, Sanwin and Neoxicam were not significantly different from that of the innovator drug Feldene^{R} (Neimmeth, Nigeria Plc) and so were judged to be bioequivalent with Feldene$^{R}_{1}$. In SIF, the PAE of the Saldin 20, Pixicam and Neoxicam were similarly judged to be bioequivalent with Feldene$^{R}_{1}$ based on the above-mentioned criteria.

The absolute drug content is presented in table 4. Amongst the various brands, drug content varied in the two media (SIF and SGF). In SGF, all the capsules contained piroxicam above the label claim at levels above the acceptable compendial standard. In SIF, only
Felxicam, Diovin, Felvin, Pixicam, Saldin, Piroxy and Feldene$_{1}$ contained piroxicam within the range of the label claim.

Table 4: Absolute Content of Multi-sourced Piroxicam Capsules Marketed in Nigeria

BRAND	Quantity of Piroxicam (mg)	
	SGF	SIF
SALDIN20	34.6 ± 1.02	17.26 ± 2.43
PIXICAM20	45.4 ± 2.01	19 04 ± 1.77
FELVIN20	40.6 ± 1.50	20.78 ± 1.98
FELXICAM	25.80 ± 1.98	21.56 ± 3.55
FELDENE$_{2}$	33.7 ± 2.29	15.13 ± 3.21
DIOVIN20	32.2 ± 1.21	20.69 ± 3.39
PIROXY	45.4 ± 4.23	20.39 ±1.45
SANWIN	37.4 ± 3.32	22.56 ± 2.57
NEOXICAM	22.8 ± 2.34	14.91 ± 3.23
MF20	43.3 + 2.56	13.80 ±2.23
FELDENE$^{R}_{1}$	21.4 + 3.00	24.86 ± 2.21

Our findings have revealed that multi-source piroxicam capsules marketed in Nigeria have varying Predicted Availability Equivalence (PAE) and content of the active ingredient. The label claim of all the samples of piroxicam used for this study is 20 mg per capsule. However, the assayed quantity of the active drug for most of the products was very much higher than the label claim. Therefore, most of the products were substandard because of the higher quantity of the active drug far above the acceptable compendial standards. This finding is in consonance with those of many studies carried out with various drugs marketed in the developing countries (Taylor *et al*, 2001; Shakour *et al*, 1997; Kibwage *et al*, 1992).

The wide variation in the content uniformity of these studied brands of piroxicam might not be deliberate, but may rather be a reflection of the poor quality control and quality assurance during manufacture (Taylor *et al*, 2001).

The fact that manufacturing procedures and formulation excipients significantly affect biopharmaceutic properties of drug formulations is well established (Babalola, 2001; Proudfoot, 1988;Jones *et al*, 1988; Ofoefule *et al*, 2001; Ofoefule *et al*, 2001; Olaniyi, 2001). This is principally the reason for stringent evaluation of multi-source preparations in terms of their comparative dissolution efficiency.

The PAE used in our study can be theoretically related to the *in vivo* data because it is based on the assumption that the degree of absorption of a drug *in vivo* is proportional to the concentration of the drug in solution and the time this solution in contact with a suitable absorption region in the gastro-intestinal tract GiT (Khan, 1975). In *in vitro* dissolution studies, this could be represented as the area under the dissolution-time curve.

Our results show that PAE values between 89-110% were not significantly different ($P<0.05$) from that of the innovator drug (Feldene^R). Therefore, only Saldin 20, Diovin 20, Sanwin 20 pixicam and Neoxicam were judged to equivalent with Feldene^R in SGF while only Saldin 20, pixicam and Neoxicam were equivalent in SIF. Predictably, dissolution media (and hence pH) affects drug release and hence bioavailability. Piroxicam is rapidly absorbed in the stomach (Pagan *et al*, 1995) and so their likely *in vitro* availability will follow the release profile of the drug formulations in SGF. It follows therefore that only Saldin 20, Diovin 20, Sanwin and Neoxicam are really bioequivalent with the innovator drug based on their statistically similar DAE values. Of these four brands, however, only Neoxicam has statistically similar absolute drug content with the innovator brand and hence complied with desirable compendial standard. The other brands have absolute drug content far above the compendial limits.

Although *in vitro* studies are not alternatives for *in vivo* studies, they do serve as cheap and rapid leads in the quality control of multi-source drugs (Ofoefule *et al*, 2001; Olaniyi, 2001). Very clearly therefore, many of the brands of piroxicam marketed in Nigeria may not strictly be switchable (substitutable) with the innovator drug. They may accomplish the therapeutic goal, but could also result in high-level toxicity (because of the higher concentration of the active constituents).

. The observed non-compliance with pharmacopoeial standards of many brands of Piroxicam capsules marketed in Nigeria calls for caution in the prescription and in dispensing of these supposed alternatives. Since many of the piroxicam brands contain excess of the active ingredient above the compendial standards, they could actually relieve pain but the potential gastrointestinal risk might be quite high and dangerous. Most of these problematic products are usually brought into the country by unauthorized importers.

We advocate a multicentre study that will screen all the brands of piroxicam in the Nigerian market with the view of listing suitable and unsuitable brands.

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