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Physicochemical equivalence evaluation of some piroxicam capsule brands in Nigeria

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Summary
Comparative chemical and pharmaceutical equivalence study on ten brands of piroxicam capsules was carried out. The aim of the study was to determine whether the brands are comparable with each other on the basis of their physico-chemical properties. The chemical and pharmaceutical equivalences of ten brands of piroxicam capsules were assessed by evaluating the uniformity of weight, dissolution rate, content identification and the chemical assay of the capsules. All the ten brands complied with British Pharmacopoiea standard for uniformity of weight. The thin layer chromatographic test for content identification showed that nine of the brands contain only piroxicam while one brand contains an additional compound apart from the labeled piroxicam. The ultraviolet procedure for content identification showed that only two brands complied with the official specification. Five brands complied with specification for the content assay of the active ingredient. However, only two brands complied with the official specification of 70%w/v dissolution at 45 minutes. The result obtained from this study showed that only the innovator brand meets all the specifications. Moreover, only five of the other brands can be regarded as chemical equivalent of the innovator. The result obtained in this study underscores the need for registration and post market surveillance of products circulating in the drug market.

Keywords: *Chemical equivalency, piroxicam, pharmaceutical equivalence, UV spectroscopy.*

Résumé
L'étude comparative avait pour but d'évaluer l'équivalence chimique et pharmaceutique sur dix genres de capsules de piroxicam. L'objectif de cette étude était de déterminer si les genres sont comparable l'un l'autre sur la base leurs propriétésphysico-chimiques. Ces équivalences étaient faite en évaluant l'uniformité du poids, le taux de dissolution, l'indentification des excipients et l'analyse chimique des capsules. Tous les 10 genres satisfaisaient le standard de la pharmacopée Britanique d'uniformité du poids. Le test de chromatographie de couches légeres pour l'identification des excipients montraient que neuf de ces genres contenaient seulement le piroxicam alors que un seul contenait un composé additionel autre que le piroxicam. La procédure ultraviolete pour l'identification des excipients montraient que seulement deux des genres satisfaisaient aux spécifications officielles. Cinq genres satisfaisaient avec la spécification de l'analyse des composantes actives. Cependant, seulement deux genres satisfaisaient la spécification officielle de 70% w/v de dissolution en 45 minutes. Le résultat obtenu de cette étude montre que seul la marque innovée satisfait toutes les spécifications. En plus, seule cinq d'autres genres peuvent etre régardés comme chimiquement équivalent á l'innovateur.Ce résultat indique le besion d'enregistrement et une surveillance des produits ou médicaments sur le marché.

Introduction

Piroxicam, 4- hydroxy- 2 methyl – N2-pyridinyl – 2H-1,2 benzothiazine – 3 – carboxamide, is a member of the oxicam derivatives of non-steroidal anti inflammatory drugs (NSAIDs) [1]. NSAIDs are a group of drugs commonly used to treat various ailments because of their analgesic, anti-inflammatory and antipyretic properties [2].

Piroxicam is one of the most widely used anti-rheumatic agents in the world. It is manufactured by a considerable number of pharmaceutical companies located in different parts of the world. As a result of this large pool of manufacturers, the drug is marketed under different brand names and there exist in the Nigerian drug market today a wide variety of piroxicam brands.

The introduction of generic drug product from multiple sources into the health care delivery system of many developing countries was aimed at improving the overall healthcare delivery systems in such countries. However, this has been accompanied by

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a variety of problems of which the most critical is the widespread distribution of fake and substandard drug products.

Although, the WHO issued guidelines for global standardization and requirements for the registration, assessment, marketing, authorization and quality control of generic drug products [3], many developing countries do not have an effective system of monitoring the quality of generic drug products being distributed within their regions.

In Nigeria, the National Agency for Food, Drug Administration and Control (NAFDAC) is the drug regulatory authority responsible for the administration and control of drugs within the nation's province. In line with the WHO guidelines, NAFDAC has standards of quality, efficacy and safety which are aimed at getting the right quality of drug products to the consumers. Both generic and branded drug products must meet these standards.

Drug products that are chemically and pharmaceutically equivalent must be identical in strength, quality, purity as well as content uniformity, disintegration and dissolution rates [4]. In Nigeria, chemical and biopharmaceutical inequivalencies have been reported for some brands of ampicillin [5] and tetracycline [6] capsules as well as metronidazole [7] tablets. However, in a study on some brands of sulphadoxine-pyrimethamine tablets, chemical as well as biopharmaceutical equivalency was observed with three out of eight brands tested, while the remaining five brands were found not equivalent [8].

The present study was aimed at determining the chemical and pharmaceutical equivalence with respect to dissolution rate of ten brands of piroxicam capsules obtained from different retail pharmacies in Ibadan, Nigeria.

Materials and methods

Reagents

Glacial acetic acid (Sigma Aldrich, U.K.), perchloric acid (BDH, Poole, U.K.), acetic anhydride (BDH, U.K.), potassium hydrogen phthalate (Aldrich Chemical, U.K.), hydrochloric acid (BDH, U.K.), brilliant green powder (Sigma Aldrich, U.K.), methanol (Sigma Aldrich, U.K.) and chloroform (Fischer, U.S.A.). Ten different brands of piroxicam capsules including the innovator brand from Neimeth International Plc under license from Pfizer Inc. U.S.A., with labelled contents of 20mg per capsule were obtained from different retail pharmacies in Ibadan.

Equipments

Ultraviolet/visible spectrophotometer (Perkin-Elmer model, Lambda 33), electrothermal melting point apparatus (GallenKamp London, model MBF 595), dissolution rate apparatus (Hanson Res Corp, California, U.S. A.).

Uniformity of weight determination

The individual capsule content from the different brands were emptied into clean dry weighing boats and weighed individually. The average weights of the capsule contents were calculated as well as their percentage deviation from the average weight.

Isolation of pure piroxicam from capsule

The content of twenty-five capsules of innovator brand of piroxicam was weighed and extracted with chloroform in an extraction tube. The organic solution was filtered and concentrated using a rotary evaporator. The concentrated extract was dried under nitrogen gas. The dried residue was recrystallised from absolute ethanol. The purity of the recrystallised piroxicam was determined using melting point, thin layer chromatography (TLC) [9] and ultraviolet-visible spectrophotometry (B.P. 1998) [9].

The extracted pure piroxicam was used as secondary standard for the chemical content determination as well as the calibration curve for dissolution rate determination. The secondary standard was not compared with a reference standard because a reference standard was not available.

Identification of Piroxicam

Thin layer chromatography (TLC) (B.P. 1998) [9]– 10mg of the secondary standard of piroxicam was dissolved in 5ml methanol and the solution was spotted on silica gel GF_{254} pre-coated plate. The sample was analyzed using the following chromatographic conditions;

Mobile phase: Toluene: acetic acid (9: 1)
Development distance: 10cm
Visualization: Ultraviolet lamp at 254nm

The procedure was repeated with equivalent weights of 10mg piroxicam from all the ten brands of piroxicam capsules.

Ultraviolet-visible spectrophotometry determination (B.P.1998) [9]: 0.02g of the secondary standard piroxicam was weighed into a 25ml volumetric flask, 20ml of methanol was added to dissolve it, 2.5ml of IM HCl was added followed by the addition of methanol to make up to volume.

The solution was diluted to produce a solution containing 0.0032mg/ml. The absorbance of the solution was recorded at 242nm and 334nm. The procedure was carried out in triplicate. The entire procedure was repeated with piroxicam capsules from the different brands.

Chemical content determination

Titrimetric method involving colour indicator end point determination was used to assay the secondary standard (B.P.1998) [9]. 0.02g of the secondary standard piroxicam was weighed into a clean dry conical flask and dissolved in 25ml mixture of anhydrous acetic acid and acetic anhydride (4:1). Two drops of brilliant green solution were added and the solution titrated with acetous perchloric acid (0.01M), which had been standardized using potassium hydrogen phthalate. A blank determination was carried out without the piroxicam pure powder. The procedure was carried out in triplicate.

The above procedure was repeated with equivalent weights of the piroxicam capsules from each of the brands using brilliant green solution as indicator.

Dissolution rate determination (B.P. 1998) [9]

a. Calibration curve for piroxicam content: 0.0001, 0.0002, 0.0005, 0.0010 and 0.0015 %w/v solutions of piroxicam in 0.1MHCl were prepared from the secondary standard. The absorbances were determined at 242nm. The absorbance readings were used to generate a calibration curve of concentration against absorbance. The regression equation for the calibration curve was $y = 222.8x + 0.0186$, $r^2 = 0.9923$.

b. Dissolution rate determination: 0.1M HCl (900 ml), which was freed of dissolved air was introduced into the dissolution vessel maintained at 37±0.5°C. One capsule was placed in the basket and lowered into the vessel containing the dissolution medium, the basket was rotated at 100 r.p.m. Samples (10ml) were withdrawn at 0, 10, 20, 30, 40, 50 and 60minutes, replaced with 10ml fresh dissolution medium after each sampling. The samples were filtered and diluted appropriately before the absorbances were measured at 242 nm. Six capsules were used from each brand.

The content of piroxicam in each sample was determined using the calibration curve.

The dissolution profiles of the different brands of piroxicam capsules were generated from the graph of the amount of piroxicam dissolved versus time. The average T_{70} (time for 70% of the active drug to be dissolved) and the amount dissolved at 45min. were obtained for each brand.

Statistical analysis

One way analysis of variance (ANOVA) was used for the statistical analysis.

Results

The country of manufacture of the piroxicam brands used in this study ranges from India, Malaysia, England and Nigeria. Most of the brands were not registered with NAFDAC as at the time of the study in 2004. The brands were labelled A – J with A being the innovator brand made in Nigeria. The pure piroxicam obtained by extraction and recrystallization from the innovator brand gave melting point of 199 – 200°C which is in agreement with earlier report of 198 - 200 °C for pharmaceutical grade of piroxicam [10]. The TLC showed the presence of one spot which had an R_f of 0.56 using toluene: acetic acid (9: 1) as mobile phase (B.P. 1998)[9] The ultraviolet spectrophotometry content identification gave a ratio of absorbance 2.3 at 334mm to 242mm for the secondary standard (Table 1). The content assay determination for secondary standard piroxicam used as reference gave 99.5% ± 0.50w/w (Table 1).

The uniformity of weight determination for all the brands showed that all the brands complied with the official specification [9]. The TLC examination of nine brands gave one spot each with R_f of 0.56, which showed the presence of piroxicam except brand J, which gave two spots with R_fs 0.56 and 0.38.

Content identification by ultraviolet spectrophotometry for the different capsules showed variations in the ratio of absorbance of the content at 334nm to 242nm. Brands A and F had values 2.4 and 2.2 respectively while the remaining eight brands had values below 2.0 (Table 1).

The result of the chemical content determination for the piroxicam brands showed that brands A, B, F, G and I contained between 95 – 105%w/w, while the remaining brands C, D, E, H and J gave piroxicam content above 105%w/w, with brand J having the highest figure of 192.44 ±1.76%w/w (Table 1).

The time for 70% w/v dissolution of the active ingredient (T_{70}), for the different brands varied. Brands A (i.e. innovator) and E gave 24.06±1.24 and 25.00±0.18minutes respectively, while the other brands did not even attain 70%w/v dissolution even at 60minutes. Brands A and E gave dissolution contents of 100.00±1.43 %w/v and 94.29±3.42 %w/v respectively at 45min (Figure 1).

Table 1: Identification test, Piroxicam content and Dissolution parameters for the ten brands of piroxicam capsules (A-J) and the pure reference piroxicam (P_{ref}) powder.

Brand	Identification Test *(Average ratio of Ultraviolet absorbance at 334nm to 242nm)*	Piroxicam content (%w/w)(Mean± S.D)	Dissolution Profile T_{70} (Minutes)	(Mean± S.D) % Piroxicam dissolved at 45min.
A (Innovator)	2.4	104.03 ± 0.87	24.06±1.24	100.00±1.43
B	0.9	97.78±0.24	>60	51.43 ±4.56
C	1.4	112.87 ± 1.63	>60	65.71± 1.34
D	0.7	117.55±0.48	>60	43.81± 0.19
E	1.4	121.71 ±0.49	25.00±0. 18	94.29± 3.42
F	2.2	99.86 ± 1.03	>60	47.62± 0.66
G	1.4	96.74±0.88	>60	18.10± 2.14
H	1.3	111.31 ± 1.13	>60	40.00± 1.01
I	1.2	99.86±0.80	>60	38.10± 4.02
J	1.2	192.44 ± 1.76	>60	20.00± 1.78
P_{ref}	2.3	99.50±0.50	-	-

P_{ref}- Pure piroxicam reference powder

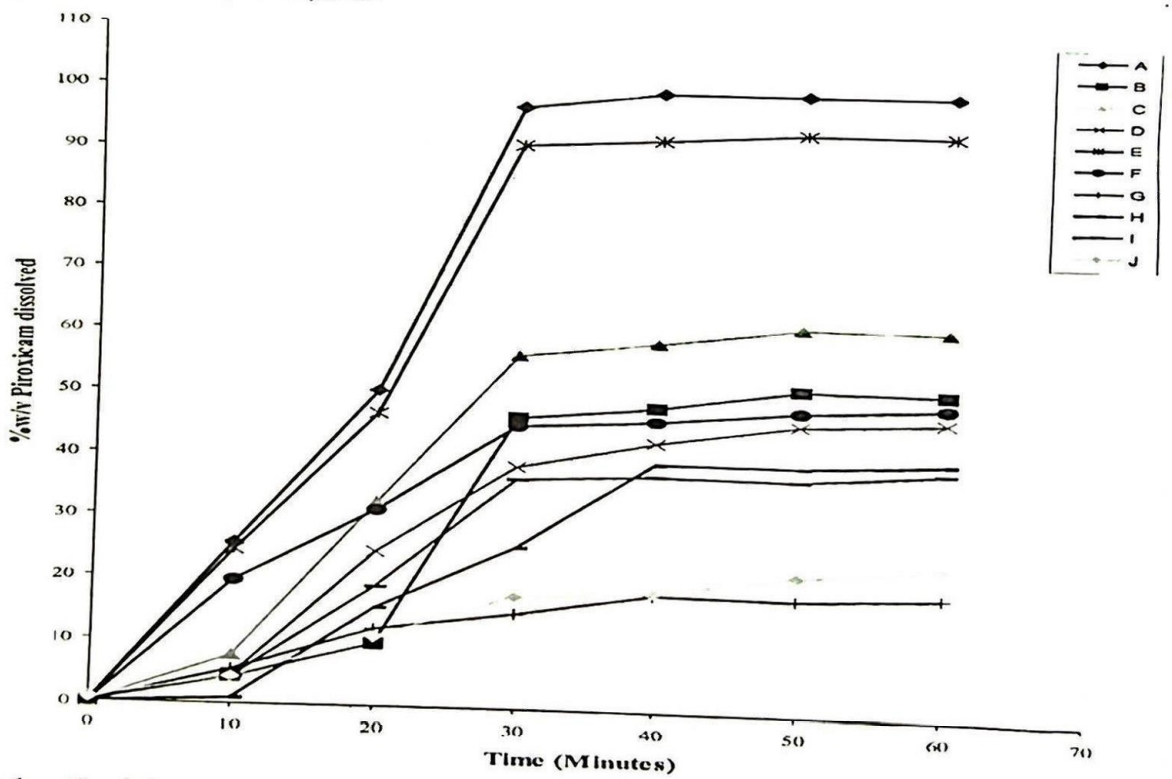

Fig. 1: Dissolution rate profiles of ten brands of piroxicam capsules in 0.1M HCI at 37 ± 0.5°C

Discussion

The pure piroxicam obtained by extraction and recrystallisation from the innovator brand complied with the official specification with a melting point of 199-200°C. Also a single spot with an R_f of 0.56 using toluene: acetic acid as mobile phase was obtained in the TLC determination (B.P. 1998) [9]. Moreover, the ultraviolet spectrophotometry content identification gave a ratio of absorbance at 334mm to 242mm of 2.3 which complies with the B.P 1998 [9] specification of 2.2 – 2.5, (Table 1). The 99.5%w/w piroxicam content obtained for the pure reference powder falls within the official specification range of 95-105%w/w (B.P.1998) [9] (Table 1). This shows that the pure piroxicam obtained by extraction from the innovator capsule dosage form can be used as a reference

(secondary standard) for other determinations. Thus, it was used as the reference pure piroxicam (secondary standard) for content identification and assay determinations as well as the dissolution rate calibration curve.

The uniformity of weight determination for all the brands showed compliance with the official specifications (B.P 1998) [9], as none of the brands deviated by up to 5% from their mean values. This indicates that the contents of the capsule in each batch within each brand are within the expected official specifications.

The R_f of 0.56 obtained with brands A – I is similar to that obtained for the reference piroxicam. This indicates the presence of piroxicam in these brands. However, brand J had an additional spot with R_f 0.38 apart from the spot of 0.56, this implies that brand J contains another compound apart from the expected active drug compound piroxicam.

The absorbance ratio at 334nm to 242nm for piroxicam using the official ultraviolet spectrophotometry content identification procedure was expected to be in the range of 2.2-2.5 (B.P.1998) [9]. However, only brands A and F with values 2.4 and 2.2 respectively, complied with this specification (Table 1). All the other brands had values below 2.0, which showed marked difference from the official specification.

The ultraviolet spectrophotometry is aimed at identifying and or proving the presence of the officially stated form of piroxicam in the samples. The ratio of absorbance readings of piroxicam solution containing 0.0032mg/ml at 334nm to 242nm is critical to the spectrum of piroxicam. Any deviation may be interpreted to mean the presence of other compounds with similar structures apart from piroxicam or that the actual content of piroxicam is not sufficient to meet the specified levels. It could even imply the presence of different polymorphic form of piroxicam apart from the official form.

Piroxicam has the ability to exhibit polymorphism [9], which is the ability to exist in one or more crystalline forms. Differences in polymorphs is characterised by differences in physico-chemical properties such as melting point, ultraviolet and infrared spectra [11,12]. Polymorphism has been reported to affect the biological action of some drugs such as chloramphenicol palmitate, aspirin, chloroquine, tetracycline [13]. The full implication of this in piroxicam is yet to be determined. The fact that only the innovator brand (A) and brand F complied with this official specification may imply that only these two brands contain the piroxicam form specified in the official books.

The official content specification for piroxicam in capsule dosage form is 95 –105%w/w. However, only 5 brands; i.e. A, B, F, G and I complied with this specification with piroxicam content within the specified range (Table 1) thus they could be regarded as being chemically equivalent. However, there was statistically significant difference in the chemical content between the innovator brand (A) and the other four brands i.e. B, F, G and I ($p < 0.05$, one-way ANOVA). On the other hand, the remaining five brands (C, D, E, H and J) did not comply with official content specifications [11]. These brands had values above the specification, i.e. between 111.31 and 192.44%w/w, with brand J having almost twice the specified value (Table 1). The obtained value for brand J is not too surprising in view of the result of the TLC identification, which revealed the presence of another compound apart from piroxicam. The piroxicam content for brands C, D, E, H and J were statistically different from the innovator brand A ($p < 0.05$, one-way ANOVA).

The chemical content determination is not specific for any of the polymorphs, as could be seen in the results obtained from chemical content determinations. The brands that failed the ultraviolet spectrophotometric identification either had more than the required piroxicam content specified in the official books (B.P. 1998)[9] or passed the piroxicam content determination test (Table 1).

The dissolution rate profile as shown in Figure 1, revealed that only the innovator brand A and brand E with T_{70} (i.e. time for 70% for the piroxicam to be released from the capsule dosage form) of 24.06 and 25.00 minutes respectively meet up with the B.P. 1998 [9] specification of 70% at 45mins. All the other brands did not comply with this specification.

Brand F that hitherto had complied with all the other specifications, i.e. piroxicam content and content identification did not comply with the dissolution rate specification. This may be due to the nature of the excipients used for the formulation process. Abdou, (1989) [14] reported that the dissolution rate of a pure drug could be altered significantly when mixed with various adjuncts during the manufacturing process of solid dosage form.

The results of the dissolution rate determinations imply that all the failed brands, i.e. B, C, D, E, F, G, H, I and J may not release a significant amount of the drug into the systemic circulation on absorption. On the other hand it could indicate differences in the physico-chemical characteristsic of the piroxicam polymorphs present in the different brands. A similar study in the United State of America

by Barone *et al* (1988) [15] on 25 brands of piroxicam capsules reported that 72% of the brands failed to meet the United States Pharmacopoeia (USP) requirement for dissolution. Another study on 85 generic products from 21 countries reported that 91% of the generic piroxicam products evaluated failed to meet the routine in vitro USP quality assurance criteria for potency and or dissolution [16]. This difference in dissolution could result in altered bioavailability and hence potency. This would result in therapeutic failure.

Moreover, the fact that most of the brands that passed the content assay determination, failed the Uv absorption ratio test may imply the limitation of the content assay determination as a means of proving the quality of drugs that exists as polymorphs such as piroxicam.

Although comparative bioavailability studies would be required to draw clinical conclusions, the failure of most of these products to meet the B.P. requirements for dissolution indicates formulation differences that could result in differences in bioavailability.

The differences in quality control parameters observed in the piroxicam capsule dosage form used in this study have implications in terms of product equivalence and standards of multisourced products available within Nigeria. The wide variation in quality of brands of piroxicam capsules studied underscores the need for registration and post market surveillance of registered products circulating in the drug market.

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