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HPLC Analysis and *In-vitro* Dissolution studies of fifteen brands of Diclofenac tablets sampled from Pharmacy stores within the Mainland area of Lagos state, Nigeria

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ABSTRACT

The present study aimed at analysis and in vitro dissolution studies of Diclofenac tablets sampled from Pharmacy stores within the Mainland area of Lagos state, Nigeria. Qualitative and quantitative parameters using visual inspection, uniformity of weight, uniformity of drug content and dissolution test were employed. High performance liquid chromatography (HPLC) method was used to analyze the amount of Diclofenac in the samples. Visual inspections of (fifteen different brands) individual tablets showed no visible physical deterioration, capping or breakage and all the samples were appropriately labelled and packaged. The Percentage content of the different Diclofenac tablets analysed was within 4.58% to 182.04%, where only two samples (13.33%) passed the USP specified range of 90 – 110%. The dissolution rates at pH 1.2 both with and without pepsin ranged from 4.0-10.0 %. At pH 6.8, there was a marked increase in dissolution (24%-134%) indicating the increase in drug release at higher pH. Only 2 of the six drugs assayed for dissolution with percent release of 63.81% and 71.76% complied with the official monograph specification that 56% -76% of the drug must be released at pH 6.8 in 4 hours. The results of this study indicate the need for periodic assessment of pharmaceutical products that are commercially available in the country.

Keywords: Diclofenac tablet, Dissolution test, High performance Liquid Chromatography, Percentage content.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequent prescribed drugs worldwide and are used for relief of inflammatory, chronic (e.g. rheumatoid arthritis, osteoarthritis and gout), and acute (e.g. headache, postoperative pain and orthopaedic fractures) pain conditions. The anti-inflammatory effects of NSAIDs are believed to be due to inhibition of leukocyte migration and the enzyme cyclooxygenase (Cox-1 and Cox-2), leading to the peripheral inhibition of prostaglandin synthesis, while the Antipyretic effects may be due to action on the hypothalamus, resulting in peripheral dilation, increased cutaneous flow, and subsequent heat dissipation (McCarberg and Gibofsky, 2012). The growing demand for NSAIDs stimulates higher level of quality control of these therapeutic substances and preparations. Diclofenac is a non-steroidal anti-inflammatory drug (NSAID), which is very effective in the management of pain, inflammation and stiffness caused by many conditions such as osteoarthritis, rheumatoid arthritis, abdominal cramps associated with menstruation, and ankylosing spondylitis (Shah, 1992). The chemical name is 2-(2, 6-dichloranilino) phenylacetic acid. It is soluble in water and hygroscopic in nature.

Diclofenac is common over-the-counter drugs that relieve pain. They do not require official prescription, before they can be purchased from the pharmacy, drug stores or drug peddler. It is mostly available in tablets (50mg, 75mg and 100mg) and injection (25mg and 75mg) as potassium or sodium salt and are readily available as pharmaceutical products in Nigeria. Many generic versions of diclofenac tablets by different manufacturers and from different countries exist today in Nigeria and hence the need to investigate their compliance to the required standards as specified in the pharmacopoeia. These tests include uniformity of weight, uniformity of drug content and dissolution etc (BP, 2010).

Counterfeit drugs (fake drugs) are substantial and growing problem, both in the developed and in the developing World. No country is free of this problem, which plagues developing and developed countries. In addition, at the global level, it was estimated that 10% of the Medicines in the World Trade were counterfeit drugs (WHO, 2012). Counterfeit and substandard medicines have been reported to contribute to the high incidences of treatment failure, morbidity, mortality and drug resistance (Caudron, 2008). The problem of counterfeit drugs is known to exist in both developed and developing countries. However, the true extent of the problem is not really known since no global study has been carried out (Taylor *et al*. 2001, Kolawole *et al*. 2002).

The present study was aimed at analysis and in vitro dissolution studies of different brands of diclofenac tablets sampled from Pharmacy stores within the Mainland area of Lagos state, Nigeria. This will enable us to ascertain the conformation of different brands to stipulated official specifications.

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MATERIALS AND METHODS

All reagents and solvents used in this study were of HPLC grade and were freshly prepared. The Diclofenac reference standard 93484-100mg, CAS; 15307-79-6, Molecular weight; 318.13g/mol, Assay; >98.5%, Product code; 101654099, potassium dihydrogen orthophosphate, sodium hydroxide pellet (99-101%), HPLC grade methanol and acetonitrile were all obtained from Merc chemicals (Darmstadt Germany). Syringe filter 0.45 µm millipore®), Mettler Toledo® AL 204 analytical balance was used in weighing, while the pH was adjusted with Jenway® pH meter, micropippete, Clifton ultrasonicator for mixing, while dissolution apparatus USP 2 (COPLEY NE6-COPD dissolution tester), was used for the In-vitro dissolution test. The double beam UV-Visible Spectrometer (PG Instruments Ltd, T80 +, S/N 15-1885-01-0094) was used to measure absorbance of the drug after dissolution. Erweka hardness tester ®, Erweka friabilator and multiunit disintegration tester electrolab® apparatus were employed in the study. An Agilent Technologies, U.SA 1200 series HPLC system with a C-8 (Zorbax Eclipse XDB RP C8 150x4.6mm, 5µm particle size) column and coupled to a UV detector was used in the chemical analysis. A mobile phase: mixture of Acetonitrile and 10mM Na Acetate in distilled water (40:60), Flow rate of 0.6ml/minute, Variable wavelength detector of 282nm, at ambient temperature (24^0C) was used for the determination of Diclofenac content in the tablets.

Sample collection

Samples were purchased from registered community Pharmacy stores within the Mainland area of Lagos state, Nigeria. The followings tablets were randomly sampled: three brands of Diclofenac potassium 50mg, three brands of Diclofenac sodium 50mg, and nine brands of Diclofenac salts (100mg). Each sample was specially coded as follows; DCLO1, DCLO2, DCLO3, DCLO4, DCLO5, DCLO6, DCLO7, DCLO8, DCLO9, DCLO10, DCLO11, DCLO12, DCLO13, DCLO14 and DCLO15 respectively. Visual inspection including manufacturing date, batch number, expiry date and NAFDAC registration number were recorded immediately on purchase (see table 1)

Sample preparation

Twenty tablets of each brand were randomly selected and weighed using the Metler Toledo analytical weighing balance, the average weight was calculated to determine the weight variation and then the equivalent weight of 50mg was derived. The 20 tablets were triturated to fine powder using a mortar and pestle, the equivalent weight obtained was measured from the powdered drug and transferred into a sample bottle in duplicates and reconstituted with 50ml of distilled water to obtain a 1mg/ml concentration of the drug solution and placed in the sonicator for about 10 minutes for agitation. A further dilution of the solution using a micropipette was made with distilled water to obtain a 10µg/ml concentration. The sample solution was filtered using the syringe filter and then transferred into a sample bottle.

High Performance Liquid Chromatography (HPLC) Analysis

The HPLC system was prepared by allowing it to run for about 30 minutes with the mobile phase. A 100mg of the diclofenac reference standard was accurately weighed into separate 100 ml volumetric flask containing 60ml of acetonitrile, shaken and sonicated to dissolve. This was made up the mark to obtain stock concentrations of 1000 µg/ml of diclofenac. Using the dilution formula ($C_1V_1=C_2V_2$), the following gradient concentrations (10, 20, 30, 40, 50 µg/ml) of standard solution was prepared. 20µL of the reference standard was introduced into the HPLC by manual injection and allowed to run (retention time of 4 minutes) and each sample was then injected into the HPLC in duplicates and allowed to run. The results were collected and the peaks obtained from the reference standards were used to plot a calibration curve, actual concentrations of samples were obtained using the regression equation and the percentage purities were calculated.

In-Vitro Drug Dissolution Studies

Preparation of different dissolution medium was carried out below:

The buffer of pH 1.2 was prepared by weighing 12g of NaCl with an analytical weighing balance, the NaCl was dissolved in distilled water, 500ml of HCl was measured using a volumetric flask and added to the NaCl solution, the mixture was made up to 6000ml with distilled water. The pH was then checked with the pH monitor.

The buffer of pH 1.2 with Pepsin was prepared by weighing 12g of NaCl and dissolving it in distilled water, 19.2g of Pepsin was also weighed and dissolved in distilled water and the two solutions were combined. 500ml of HCl was measured using a volumetric flask and added to the mixture, it was then made to 6000ml and the pH was monitored using the Metler Toledo pH meter.

The buffer of pH 6.8 was prepared by weighing 172.8g of Disodium Hydrogen Phosphate and 68.7g of Potassium Dihydrogen Phosphate, both salts were dissolved in distilled water and then made up to 6000ml. The buffer of pH 7.4 was prepared from two solutions:

I: Disodium hydrogen phosphate 656.205g was dissolved in sufficient distilled water to produce 5500 ml.
II: The KH_2PO_4 45.36 g was dissolved in sufficient distilled water to produce 1000 ml. Then 5100ml of solution I and 900 ml. of solution II were mixed to produce 6000ml buffer and the pH was adjusted using distilled water.

Dissolution studies of Six brands of Diclofenac (100mg tablets)
A tablet of the 6 brands (DICLO7, DICLO9, DICLO10, DICLO11, DICLO12, and DICLO13) of Diclofenac was dropped into the dissolution vessels simultaneously as the equipment timer was started. 5mls of the dissolved drug sample in solution was taken out at a time interval of 45minutes; the portion taken out was replaced with 5ml of the medium at each interval to maintain equilibrium in the system (Akinleye et al., 2012).
The 5ml extracted at each time interval for the different brands was then filtered using syringe filter into labelled sample bottles. The filtrate was transferred into the cuvette of the UV spectrometer and the absorbance was determined. Proper dilutions were made when the pH of the medium became basic to allow for the increased concentration and to ensure that Beer-Lambert's law was followed. The readings obtained were recorded and used in the plotting of calibration curve; the concentration was obtained from the equation of the line and used in the calculation of percentage drug release.

RESULT AND DISCUSSION
The results of visual inspection of the samples showed that all of the samples have on their packaging, brand name, full address/country of origin, batch number, expiry/manufacturing dates, strength and dose/advice to see the physician. Physical inspection of individual tablets showed no visible physical deterioration, capping or breakage. The result of the visual inspection showed nine (9) of the brands were manufactured from India which accounts for 60% of the samples and thirteen (13) out of fifteen (15) brands were imported into Nigeria, while only two (2) brands was manufactured in Nigeria (Table 1).
The Calibration curve reveals the linearity in the concentration range of 0.5 to 40µg/ml with correlation coefficient (r) of 0.996 and the linear regression equation that was used for the calculation was Y= 21.61x (Figure 1). The Percentage content of all the different Diclofenac tablets analysed was within 4.58% to 182.04%, where only two samples (DICLO7 and DICLO11) passed the USP standard range of 90 – 110 %: Table 2. From the studies carried out with HPLC analysis, all of the Diclofenac 50mg strength used falls below USP stipulated percentage purity range. Since all the drugs were analysed before the expiry dates, some other factors affecting the drug may include the storage conditions under which the drugs had been kept. For the Diclofenac 100mg strength, only two out of the nine brands used for the analysis passed the USP stipulated range.
Dissolution test is an important parameter for assessing drug release from pharmaceutical dosage forms and as an indirect method of measuring drug availability (Mbah *et al*., 2012). The dissolution test was investigated at different pH. The pH of 1.2 was used both with and without pepsin in order to mimic the gastric environment of the stomach, the pH of 6.8 mimics the pH of the duodenum while studies at pH 7.4 was aimed at mimicking the pH of the Jejunum. Diclofenac should be absorbed from the small intestine which comprises the duodenum and the jejunum, because of the slight alkaline environment which would enable it to react with the slightly acidic diclofenac. At pH of 1.2, the result obtained for the dissolution shows that no significant dissolution occurred with highest percent drug release being at 10% which shows that the drug was not released in the acidic environment (figure 2-7). This can be corroborated with the work of Sabnis *et al* (1997), where the low release rate of diclofenac sodium (pKa = 4) , in 0.1 M HCl (pH 1.5) has also been attributed to i ts low solubility in acidic medium, hile the drug is known to be soluble in waterand basic media (Mitr evej *et al*., 2001). The release of drug reduces when the drug is tested at the same pH with pepsin, most of the drugs did not dissolve in this medium with the highest dissolution achieved being 4% (Figure 2-7). At the pH of 6.8, there was a marked increase in the dissolution of diclofenac with percentage release for the brands ranging from 24%-134% while the official monograph specifies that 56% - 76% of the drug must be released at 4 hour. The increase in drug release at higher pH can be attributed to pH dependent solubility of diclofenac sodium. As the pH increases, the solubility of diclofenac sodium increases and hence increases in drug release (Khan, 2012). Only 2 of the drugs used conformed to the specification which include DCLO7 (figure 4) with a percentage release of 71.76% and DCLO10 (figure 2) with a percentage release of 63.81%. At pH 7.4 the percentage release obtained ranged from 6-139%. However, none of the drugs analyzed was within the stated official range. DCLO10, DCLO11 and DCLO12 showed dissolution of more than 100% after 4 hours (Figure 2,6 and 7) suggesting that the drugs would be completely absorbed from the jejunum which is the appropriate pH for this, but also indicates that the drugs contain more than 100mg of the drug. The erratic dissolution result exhibited by these drugs could be as a result of storage conditions,

granule size, compression force, moisture content and other formulation parameters. The results of this study indicate the need to regulate the brands of enteric coated Diclofenac being produced and which are being sold in the country through evaluation of quality control parameters in order to avoid counterfeiting and drug adulteration.

CONCLUSION

Visual inspections showed all the samples were appropriately labelled and fairly packaged. Only two of the brands analyzed complied with the respective USP specifications (90 – 110%) for percent drug content for Diclofenac tablets. Also, only two out of six drugs assayed for dissolution complied with the specification of the official monograph. There is need for periodic assessment of pharmaceutical products that are commercially available in the country.

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Table 1: Visual Inspection of Diclofenac tablets samples

Brand code	Country of manufacture	Batch number	Manufacture date	Expiry date
DICLO1	Switzerland	K0684	June 2015	July 2107
DICLO2	Malaysia	BF06578	June 2015	June 2018
DICLO3	India	K402	Oct 2014	Sep 2017
DICLO4	India	7551501	May 2015	April 2018
DICLO5	India	SE01276	Sept 2015	August 2018
DICLO6	India	JD1502	Nov 2015	Oct 2018
DICLO7	Switzerland	S0127	April 2015	March 2020
DICLO8	India	H202	August 2013	July 2016
DICLO9	China	140623	May 2014	June 2017
DICLO10	India	1R0011403	July 2015	June 2018
DICLO11	Nigeria	4D896001	April 2014	March 2017
DICLO12	India	LTC155	Dec 2014	Nov 2017
DICLO13	Israel	1312040	Dec 2013	Dec 2018
DICLO14	India	M302	Dec 2013	Nov 2016
DICLO15	Nigeria	0402	April 2016	March 2018

Note: DICLO1 to DICLO6 is 50mg and DICLO7 to DICLO15 is 100mg

Table 2: Percentage content for the different Diclofenac brands using HPLC

Brand code	Average weight(mg)	Nominal conc (µg/ml)	Calculated conc (µg/ml)	Percentage content	Remarks USP
DICLO1	322.55	10	5.074	50.74	Failed
DICLO2	221.30	10	0.919	9.19	Failed
DICLO3	195.30	10	0.975	9.76	Failed
DICLO4	214.95	10	0.619	6.19	Failed
DICLO5	132.85	10	1.020	10.20	Failed
DICLO6	194.70	10	0.457	4.58	Failed
DICLO7	301.80	10	10.039	100.40	Passed
DICLO8	363.17	10	4.461	44.62	Failed
DICLO9	250.74	10	7.726	77.26	Failed
DICLO10	190.26	10	18.203	182.04	Failed
DICLO11	350.46	10	10.9081	109.08	Passed
DICLO12	383.47	10	3.880	38.80	Failed
DICLO13	319.58	10	4.193	41.93	Failed
DICLO14	370.20	10	6.527	65.27	Failed
DICLO15	348.50	10	6.586	65.86	Failed

Note: DICLO1 to DICLO6 is 50mg and DICLO7 to DICLO15 is 100mg

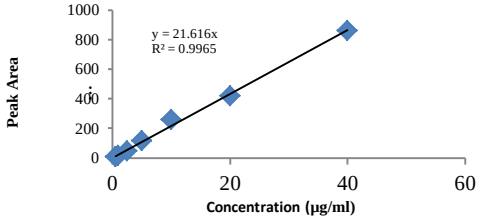

Figure 1**:** Calibration curve for Diclofenac standard

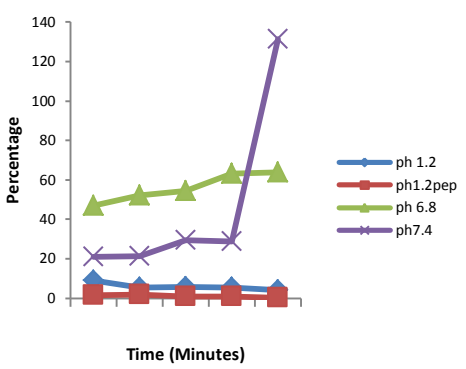

Figure 2: Percentage drug release against time for DICLO10 at different pH

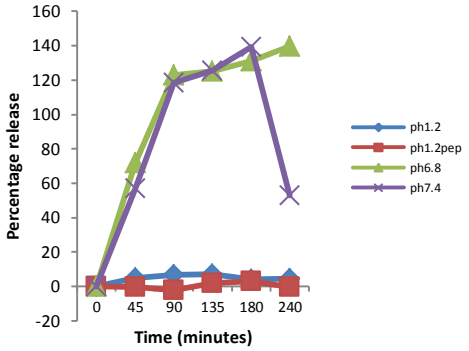

Figure 3: Percentage release against time for DICLO9 at different pH

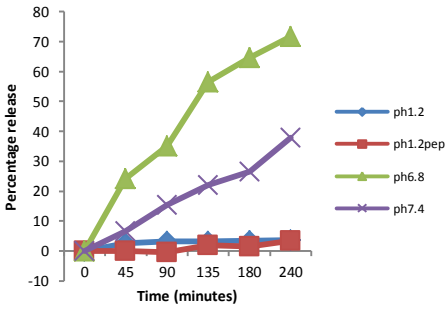

Figure 4: Percentage release against time for DICLO7at different pH

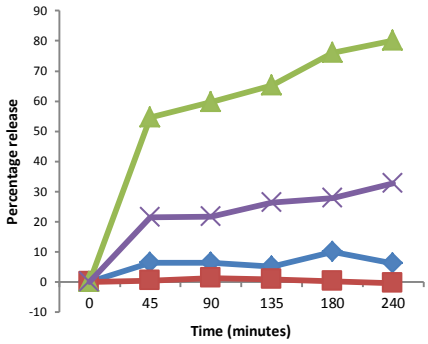

Figure 5: Graph of percentage release against time for DICLO13 at different pH

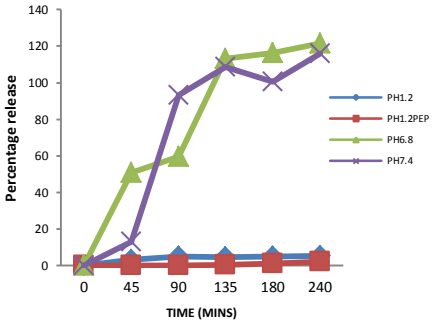

Figure 6: Percentage release against time for DICLO12 at different pH

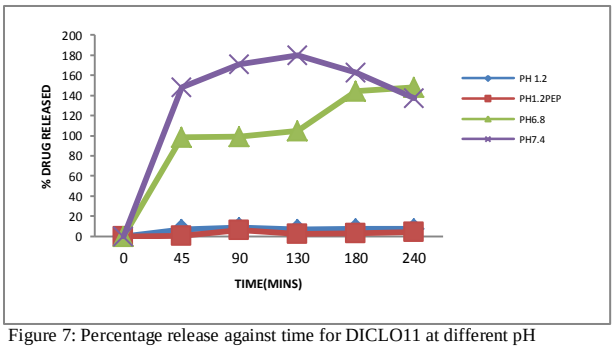

Figure 7: Percentage release against time for DICLO11 at different pH

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