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Original Research

Quality assessment of different brands of diclofenac tablets marketed in Ilorin metropolis: a pharmaceutical and public health perspective

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ABSTRACT

Background: The quality of a medicinal product is an important factor for its safety and efficacy. Poor-quality medicines are a major impediment to improvements in public health. This study assessed the pharmaceutical quality of different brands of diclofenac (DCF) tablets in Ilorin metropolis.

Methods: Four randomly selected brands of diclofenac potassium tablets (coded: DCF-A, DCF-B, DCF-C and DCF-D) were obtained from pharmaceutical outlets, and quality parameters were evaluated according to Pharmacopeial methods. The potency of tablets was determined spectrophotometrically based on the measurement of maximum absorbance at a wavelength of 276 nm in doubly distilled water.

Results: Method validation according to the International Council for Harmonization guidelines showed acceptable sensitivity (limit of detection of 0.3886 µg/mL and limit of quantification of 1.1775 µg/mL), precision (% relative standard deviation range of 0.72 – 1.54), accuracy (% recovery range of 98.9 – 101.3). Average contents of active diclofenac were 45, 98, 103 and 105% for DCF-A, DCF-B, DCF-C and DCF-D respectively. DCF-A brand was not only substandard but falsified based on British Pharmacopoeia potency specification range of 95 – 105%.

Conclusion: A substandard and falsified brand of diclofenac tablets was detected. Drug regulatory authority must ensure periodic post-registration surveillance of licensed pharmaceutical products marketed in the country to secure the health and safety of the populace.

Keywords: diclofenac tablets, spectrophotometric analysis, quality, substandard, falsified medicines

INTRODUCTION

The existence of substandard and falsified medicines poses a serious threat to public health[1]. According to World Health Organization (WHO), one in ten medicines in low- and middle-income countries are estimated to be substandard or falsified, and they affect every region of the world with medicines from all major therapeutic categories involved[2]. Medical products which are authorized but fail to meet either their quality standards or their specifications or both are said to be substandard. On the other hand, medical products that deliberately or fraudulently misrepresent their identity, composition or source are considered to be falsified[3]. Diclofenac is one of the most commonly used nonsteroidal anti-inflammatory drugs (NSAIDs) of the phenylacetic acid class with anti-inflammatory, analgesic, and antipyretic properties[4,5]. The reason for the frequent prescription can be attributed partly to the wide range of diclofenac products of varied pharmacokinetic properties and dosing regimens[4,6]. It is available as a potassium salt (immediate-release) or sodium salt (delayed-release) and in a number of administration forms which can be given orally, rectally or intramuscularly for the treatment of a wide range of acute and/or chronic pain conditions respectively. The potassium salt is more water-soluble and considered to provide more rapid dissolution, and faster absorption than the sodium salt and approximately 50% of the absorbed dose is available due to the first-pass metabolism[4,7]. While diclofenac is a non-selective inhibitor of both isoforms of the cyclooxygenase enzyme (COX-1 and COX-2), it has a moderate preference for blocking the COX-2 receptor, although not as selective as celecoxib[8]. It may also interact with the lipoxygenase enzyme pathway, and with the release and reuptake of arachidonic acid[9,10]. Efficacy of diclofenac is equivalent to that of the many newer and established NSAIDs with which it has been compared. It is 3-1000 times more potent than other NSAIDs on a molar basis in regard to inhibition of cyclooxygenase activity[11]. Diclofenac is used in the treatment of pain in rheumatoid arthritis and other musculoskeletal conditions, migraine, fever, acute gout and post-operative pain[12]. It also has an established role in oncological practice in the treatment of cancer-related pain[13]. Diclofenac possesses an acidity constant of 4.0 and a partition coefficient of 13.4. The major structural components of the diclofenac molecule were developed based on structure-activity relationships of other NSAIDs[8] and included a phenylacetic acid group, a secondary amino group, and a phenyl ring with two chlorine atoms in the ortho position to force maximum twisting of the phenyl ring in relation to the rest of the molecule (Figure 1).

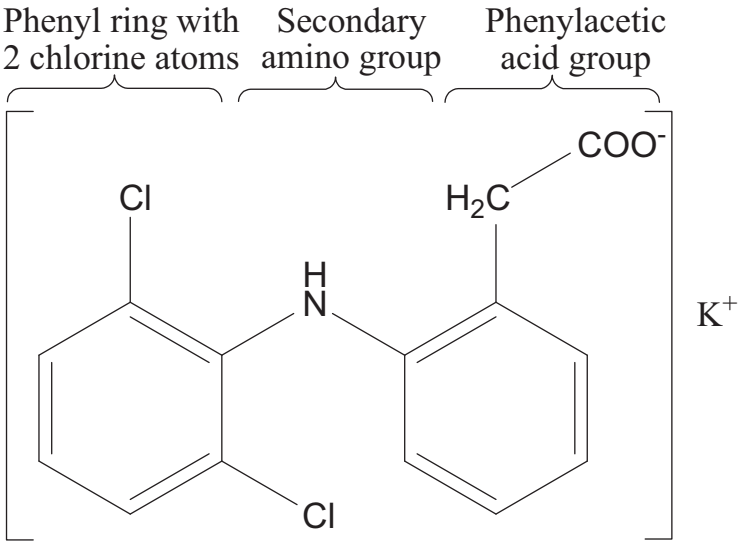

Figure 1: The chemical structure of diclofenac potassium

Substandard and falsified medicines pose a serious threat to patient safety and public health, particularly in Africa. Data on the quality assessment of drugs have focused mainly on antimicrobial agents, most especially antiretroviral, antibacterial, and antimalarial medications[14,15]. Between 20–33% of people across the globe live with a painful musculoskeletal condition. In the 2017 Global Burden of Disease report, musculoskeletal conditions were the highest contributor to global disability and accounted for

16% of all years lived with disability. Lower back pain remained the single leading cause of disability and arguably the most prevalent musculoskeletal condition in Africa[16]. To our best knowledge, only a few studies have evaluated the quality of essential anti-inflammatory medications used for the treatment of these musculoskeletal conditions[17-19]. This study assessed the pharmaceutical qualities of widely used and available brands of diclofenac potassium tablets in Ilorin metropolis, North Central Nigeria and its implication to the patient and public health safety.

MATERIALS AND METHODS

Instruments

GS-UV61PC double beam spectrophotometer (serial No: UQC1212006 by General Scientific Hong Kong Limited), Roche friabilator (Erweka Apparatebau, Germany), Vernier calliper, Monsanto hardness tester (Monsanto Chemical, USA), USP disintegration apparatus (Electolab ED-2L), water distiller (model: Basic/PH4 Pure-Hit Still). All weights were taken on an electronic analytical balance (PA214, Ohaus, USA).

Collection of sample and reference standards

According to Medicine Quality Assessment Reporting Guidelines[20], we prospectively purchased samples of four randomly selected brands of commonly available diclofenac potassium tablets marketed in the country from prominent retail and wholesale pharmacy outlets in different locations of Ilorin. Two batches per brand of 100 tablet samples were collected anonymously and randomly between May 14 and July 30, 2018, from a total of 8 outlets. Visual inspection and registration verification of all samples were conducted in compliance with the WHO recommendations[21]. The samples were identified by the name of the company, batch number, National Agency for Food and Drug Administration and Control (NAFDAC) registration number, manufacturing and expiry date, etc. The samples were then coded as DCF-A, to DCF-D. Diclofenac analytical reference standard was purchased from Pawar Supplier Karad, Maharashtra, India.

Thickness test

Ten randomly selected tablets were used by placing each tablet in-between the teeth of the vernier calliper and was gently screwed together to avoid breaking until the tablet was held firmly and the values on the calliper measured[22].

Hardness (Crushing strength) test

Ten tablets were randomly chosen, and each was placed between two anvils of the Monsanto hardness tester to allow for the crushing strength applied diametrically, which just caused the tablet to break, to be recorded[23].

Friability test

Twenty (20) tablets were randomly selected, dusted and weighed (W_o). They were then placed in a Roche friabilator drum after which the apparatus was switched on to revolve at a speed of 25 rpm dropping the tablets through a distance of 6 inches with the timer set for 4 minutes. At the end of this operation, the tablets were removed from the friabilator, dedusted and reweighed (W). Tablets that broke up were rejected before reweighing[24]. The percentage loss in weight (friability) was calculated as $(W_o - W)/W_o*100\%$.

Disintegration time test

Disintegration time of six randomly selected tablets from each batch was determined using the disintegration test apparatus-double unit. Briefly, a single tablet was placed in each of the six tubes of the basket. The basket rack was immersed in a bath of distilled water (as the disintegration medium) maintained at 37±2°C in a 1L beaker. The apparatus was operated (to move the basket assembly containing the tablets), and the time required for all the six tablets to break into particles and pass into the disintegration medium was recorded[25].

Weight uniformity test

Twenty (20) tablets were randomly selected from each batch and weighed individually, and the average weight (W) was calculated. The calculated average weight (W) was used to compute the lower and upper limits at the % difference allowed (i.e. A%) using the following equation: $W \pm (A\%*W)$. Furthermore, the lower and upper limits at double the % difference allowed were calculated: $W \pm (2*A\%*W)$. Weights of the individual

tablets were then compared with the upper and lower limits calculated at the % difference and double the % difference allowed. According to the United States Pharmacopoeia, the weight variation range of ±7.5% (for tablets with an average weight of 130-324 mg), or ± 5% (for > 324 mg average tablets weight) was used. The tablets comply with the specification if no more than two tablets (2 out of the 20 tablets) differ from the average weight by the aforementioned % difference, and no tablet differs from the average weight by double the % difference[25].

Identification test

The identification test for the active diclofenac content of the tablets was done by preparing a 20 µg/mL test solution of the sample in doubly distilled water and scanning in UV spectrophotometer from 350 nm to 200 nm to determine the wavelength of maximum absorbance (λ_{max})[17].

Preparation of standard stock and working standard solutions

100 mg of diclofenac analytical reference standard powder was accurately weighed and transferred into 100 mL volumetric flasks, dissolved and made up to mark with doubly distilled water to obtain the standard stock solution (1000 µg/mL). A 10 mL of the stock solution was pipette out and made up to 100 mL to get the secondary or working standard solution (100 µg/mL).

Generation of the standard calibration curve

From the secondary standard solution, aliquots of 2.5, 5, 7.5, 10, 12.5, 15, 20 and 22.5 mL were transferred to a series of 50 mL volumetric flasks and volume in each flask was adjusted to 50 mL with doubly distilled water to produce 5, 10, 15, 20, 25, 30, 40 and 45 µg/mL solutions. Each of the concentration levels was prepared in triplicates, and the absorbances at 276 nm were recorded. The standard calibration curve was plotted by using average (n=3) maximum absorbance versus concentration.

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were determined based on the standard deviation of the y-intercept and the slope of the three independent calibration curves as specified by the International Conference on Harmonization (ICH) guidelines[26]. Diclofenac LOD and LOQ were calculated as $3.3*\sigma/S$, and $10*\sigma/S$, respectively, where σ is the standard deviation of *y*-intercept and *S* is the slope of the calibration curve.

Method validation

The method was validated according to ICH guidelines for validation of analytical procedures[26]. Typical validation characteristics such as linearity, range, precision, robustness, ruggedness, and accuracy were evaluated.

Intra-day and inter-day precision

In intraday precision, the test solutions were analyzed in triplicates within 18 hours (i.e. at 8 am, 2 pm and 8 pm), while intermediate precision was evaluated by carrying out the assay on three consecutive days. Observed mean concentrations, standard deviations and % relative standard deviation (RSD) were calculated.

Robustness and ruggedness

Robustness was carried out to evaluate the influence of a small but deliberate variation in the spectrophotometric condition for determination of the active pharmaceutical ingredient of the tablet formulation. Robustness data for variations (±1 nm) in the detection λ_{max} of 276 nm were noted. The ruggedness of the method was determined by carrying out an analysis of test solutions by two different analysts in the same laboratory and also using different UV spectrophotometers. The respective absorbances, observed concentrations and %RSD values were computed.

Accuracy (%Recovery)

Accuracy of the method was ascertained using the reference standard and standard addition to drug product (i.e. sample) methods at 80, 100 and 120% concentration levels of the 20 µg/mL test solution (i.e. 16, 20 and 24 µg/mL) according to the ICH guideline[26]. Each of the pre-quantified concentration levels was spiked with approximately 3 µg diclofenac analytical reference standard in the standard addition method. Absorbances were measured at 276 nm, and the concentrations after addition were determined. The test was

performed in triplicates. The % recoveries of known and added amount to drug product after addition were calculated.

Assay of diclofenac in the tablet formulation

Twenty tablets from each of the batch of the brands were weighed and crushed. A quantity of the obtained powder equivalent to 100 mg diclofenac was accurately weighed and dissolved in a volumetric flask with sufficient doubly distilled water to produce 100 mL (C_1). The solution was allowed to stand for about ten minutes to allow for the complete dissolution of the active ingredient after which it was filtered. 10 mL of C_1 solution was then diluted to 50 ml with distilled water to obtain C_2. A 5 mL solution of C_2 was transferred into a 50.0 mL volumetric flask and diluted with distilled water to give C_3 with a concentration of 20 µg/mL. The absorbance of the solution (C_3) was then measured with a UV spectrophotometer using a 1 cm layer quartz cuvette at the λ_{max} of 276 nm.

Statistical analysis

Results are presented as mean (standard deviation) and %RSD. Statistical comparison was made by one-way analysis of variance (ANOVA). Results were analyzed using SPSS 16.0 software. Significant differences were set at P values < 0.05. Three quality categories were defined using the ratio of measured to expected content of the active diclofenac in the sample. A ratio of 95 - 105% indicated good quality based on the BP specification. A ratio of 85 - 95% or 105 - 115% indicated low quality, and a ratio of less than 85% or greater than 115% indicated substandard and/or falsified product[27].

RESULTS AND DISCUSSION

Descriptive characteristics of study tablets

With the exception of DCF-C, which was uncoated, the remaining three brands were coated tablets. DCF-B and DCF-C have three years of shelf lives. DCF-D had the shortest shelf life of two years with DCF-A having the longest shelf life of four years, as summarized in Table 1.

Table 1: Descriptive features of diclofenac potassium tablets studied brands

Sample code	Batches	NAFDAC numbers	Manufactured countries	Manufactured dates	Expiry dates	Description
DCF -A	5014 5015	A4-5284	India	11/2015 11/2015	10/2019 10/2019	Pink, film -coated
DCF -B	7069 7070	A4-8395	India	04/2017 04/2017	03/2020 03/2020	Orange, film-coated
DCF -C	04 17	A4-0968	Nigeria	01/2018 07/2017	12/2020 06/2020	Green, uncoated
DCF -D	513 889	04-0025	Turkey	10/2017 11/2017	09/2019 10/2019	Brown, sugar-coated

Abbreviation: NAFDAC - National Agency for Food and Drug Administration and Control

Hardness and thickness tests

No correlations were observed between tablet thickness and hardness for all the brands ($P > 0.05$), but significant batch to batch variations in tablet thickness was found in DCF-B (P, 0.016) and DCF-C (P, 0.035). This was corroborated by the average % variations in tablet thickness of the two batches of 8.88 for DCF-B and 8.48 for DCF-C compared to 4.56 of DCF-A and 0.77 of DCF-D. Tablet thickness is controlled within ± 5% variation of the standard value, depending on the size of the tablet for consumer acceptance of the product, and to facilitate packaging. Similar variation in batches was observed in tablet hardness of DCF-D (P, 0.004) as depicted in Table 2. Crushing strength of 4-8 kg for uncoated tablets and 10-20 kg for coated tablets are acceptable[25]. Hence, DCF-C with an average tablet crushing strength value of 3.25 kg failed the hardness test.

Table 2: Comparison of tablet hardness and thickness within and between batches

Brand	Batch	Thickness (mm)	Hardness (kgF)	P-value
DCF-A	5014	6.32 (0.49)	7.95 (0.83)	0.228
	5015	6.56 (0.09)	8.55 (0.86)	
	P-value	0.158	0.131	
DCF-B	7069	6.14 (0.58)	10.53 (0.97)	0.452
	7070	5.52 (0.46)	9.44 (1.46)	
	P-value	0.016	0.066	
DCF-C	OS04	6.55 (0.55)	3.40 (1.07)	0.812
	OS17	7.15 (0.62)	3.10 (0.93)	
	P-value	0.035	0.513	
DCF-D	513	5.94 (0.03)	6.48 (0.53)	0.262
	889	5.94 (0.06)	4.75 (1.59)	
	P-value	0.928	0.004	

Results are presented as mean (standard deviation)

Friability and disintegration tests

Friability and disintegration test results are presented in Table 3. A friability value of less than or equal to 1% is acceptable. The only uncoated DCF-C brand of tablets failed friability test with values of > 1% for both batches. This was not unexpected as its hardness values of 3.40, and 3.10 kg were below the recommended limit, an indication that friability is a property that is related to tablet hardness. Surprisingly, DCF-C tablets also had the highest average disintegration time of 18.43 (3.47) minutes for both batches, contrary to the expectation of lower disintegration time because of its higher friability values. The film-coated DCF-A tablets demonstrated the shortest disintegration time of 5.72 (4.72) minutes. Thus, all the four brands passed the disintegration time test as values for DCF-A, DCF-B and DCF-D were also far below the stipulated 30 minutes for the uncoated and film-coated tablets and 1 hour for the sugar-coated tablets[25].

Table 3: Comparison of friability and disintegration test results between brands

Sample code	Batch	Friability (%Loss)	Disintegration Time (min)
DCF - A	5014	0.48	2.38
	5015	0.00	9.06
DCF - B	7069	0.12	6.49
	7070	0.19	10.46
DCF - C	OS04	4.71	15.97
	OS17	1.45	20.88
DCF - D	513	0.00	12.46
	889	0.00	16.37

Weight uniformity test

Although there was no statistically significant batch to batch variations in the tablet average weights for all the brands ($P > 0.05$), batch OS04 of DCF-C failed the test as a total of three tablets have their individual weights outside the first 920 – 1,017 mg limit (2 tablets) and second 872 – 1,065 mg limit (a tablet) as presented in Table 4. Coated (DCF-A, -B and -D) tablets are exempted from these requirements[25].

Table 4: Comparison of weight variation test results between batches within brand

Sample code	Batches	Average weight (SD) mg	P-value	Composite average weight (lower - upper limits) mg
DCF - A	5014	987 (19)	0.865	987 (938 - 1,036)
	5015	988 (17)		989 (939 - 1,038)
DCF - B	7069	1,289 (48)	0.770	1,295 (1,230 - 1,359)
	7070	1,284 (50)		1,285 (1,221 - 1,349)
DCF - C	OS04	969 (46)	0.145	969 (920 - 1,017)
	OS17	989 (42)		989 (940 - 1,039)
DCF - D	513	505 (14)	0.702	504 (479 - 530)
	889	506 (9)		507 (481 - 532)

Abbreviations: SD: - standard deviation

Identification test

Spectra of solutions of all the test samples matched the spectrum of a solution of the analytical reference standard containing an equivalent concentration of diclofenac by exhibiting maximum absorbance at λ_{max} of 276 nm as presented in Figure 2 (A - E), an indication of the fact that the tablets contain diclofenac as the active pharmaceutical ingredient.

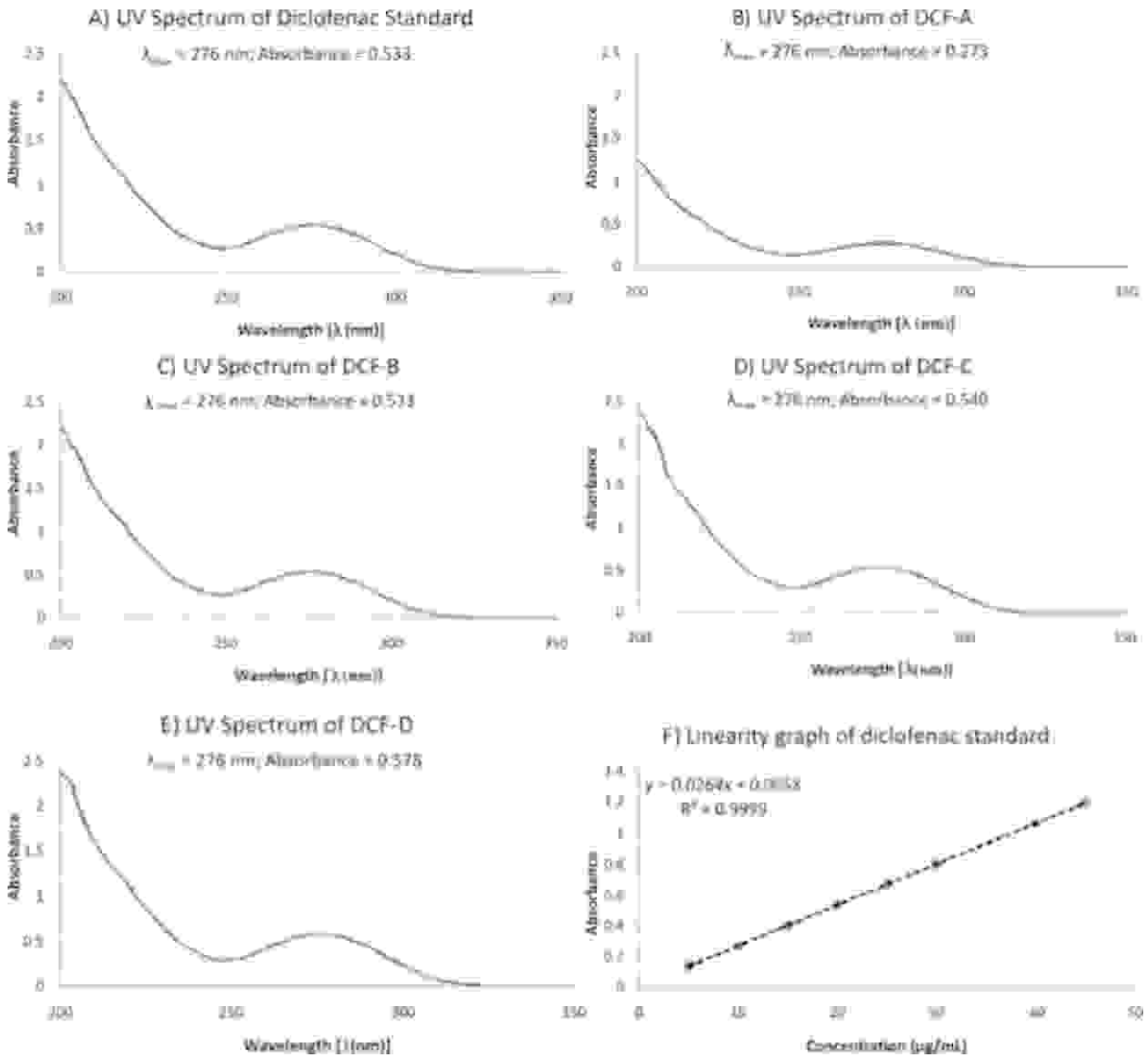

Figure 2: Ultra-violet (UV) spectrum of A) diclofenac reference standard; B) DCF-A; C) DCF-B; D) DCF-C and E) DCF-D tablets showing maximum absorbance at the wavelength of 276 nm, and F) the linearity graph of diclofenac reference standard

Linearity and range

All the eight standard calibration points used in the determination showed good recovery, which range from 99.4 to 100.4% with average %RSD of 0.8 (Table 5). The calibration curve of diclofenac reference standard's absorbance versus concentration showed good linearity in the concentration range of 5 – 45 µg/mL with correlation coefficient (r^2 = 0.9999) as display in Figure 2F. With the exception of Dhokale *et al.* (2016) that reported 10 – 50 µg/mL[28], previous studies with UV detection at 276 nm reported lower range of 1 – 30 µg/mL (17,27). The regression equation, $y = 0.0264x + 0.0058$ was obtained.

Table 5: Calibration curve absorbance (λmax of 276 nm) at different aliquots

S/No.	Concentration (µg/mL)	Absorbance (SD)	%RSD	Observed concentration (µg/mL)	% Recovery
1	5	0.138 (0.001)	0.72	5.01	100.15
2	10	0.268 (0.006)	2.40	9.94	99.44
3	15	0.400 (0.005)	1.25	14.93	99.55
4	20	0.536 (0.001)	0.19	20.08	100.42
5	25	0.668 (0005)	0.69	25.08	100.33
6	30	0.798 (0.006)	0.69	29.99	99.98
7	40	1.059 (0.003)	0.29	39.91	99.77
8	45	1.194 (0.002)	0.17	44.99	99.99

Abbreviations: SD: - standard deviation; RSD: - relative standard deviation

Sensitivity

Sensitivity is expressed by means of sensitivity index known as Sandell's sensitivity (S) which represents the number of µg of diclofenac per mL of the solution having an absorbance of 0.001 for a pathlength of 1 cm. the calculated value of S is 0.0373 μgcm^{-2} using the obtained molar absorptivity value of 8,952 $Lmol^{-1}cm^{-1}$. The LOD and LOQ values of 0.3886 µg/mL and 1.1775 µg/mL were determined, respectively. The high value of molar absorptivity and the low value of S and LOD indicate the high sensitivity of the method. The method was also approximately two-fold more sensitive compared to 0.6510 µg/mL and 2.172 µg/mL previously reported by Gunji *et al.* (2012) study which used 5M urea solution as the assay solvent[29]. The calculated specific absorbance for diclofenac potassium was of 268 ± 3.

Method validation

Precision, Robustness and Ruggedness

The study method demonstrated good repeatability with a 98.4 – 99.9% recovery and an average %RSD of 0.79. Similarly, intermediate precision was excellent with a recovery range of 99.1 – 99.9% and an average %RSD of 0.98, as presented in Table 6. Result of the small variation in the analytical detection λ_{max} of 276 nm showed good recoveries of 100.4 and 99.8% at 275 and 277 nm respectively with %RSD of < 2. Similarly, the result of analysis using different analysts and instruments indicated a good recovery range of 98.4 to 99.1% with the average %RSD of < 2 (Table 6).

Table 6: Method Precision, Robustness and Ruggedness

Parameters	Settings	Concentration (SD) µg/mL	% RSD	% Recovery
Precision				
Time (h)	0	19.98 (0.14)	0.72	99.90
Intraday	6	19.68 (0.02)	0.11	98.40
	12	19.84 (0.31)	1.54	99.20
	0	19 .98 (0.14)	0.72	99.90
Interday	24	19.97 (0.27)	1.33	99.85
	48	19.81 (0.17)	0.88	99.05
Robustness				
Wavelengths (nm)	275	20.08 (0.07)	0.33	100.40
	277	19.96 (0.17)	0.86	99.80
Ruggedness				
Analysts	I	19.81 (0.17)	0.88	99.05
	II	19.72 (0.30)	1.54	98.60
Instruments	UV61PC	19.81 (0.17)	0.88	99.05
	715N	19.68 (0.10)	0.48	98.40

Abbreviations: SD: - standard deviation; RSD: - relative standard deviation

Accuracy (%Recovery)

Accuracy is the closeness of test results to the true value and analysis of the diclofenac reference standard is one of the best ways of demonstrating it. Accuracy result using reference standard indicated recovery range of 98.9 - 99.9% with an average %RSD of 0.90. Recovery range of 100.2 - 101.3% was obtained using the standard addition method with an average %RSD of 0.88, as depicted in Table 7. The accepted limits of recovery are 98 - 102% and the obtained results for both methods were within the stated specification. The results showed excellent recoveries (100.2 - 101.3%) of the added amount of known to the test sample obtained at each level, indicating that the method was accurate and that the excipients employed in the formulation of the tablets did not interfere with the analysis by the proposed method. The method of analysis was therefore precise, accurate and robust. A summary of all the validation parameters is presented in Table 8.

Table 7: %Recovery of the analysis of reference standard and added amount to sample

Methods	Concentration (SD) µg/mL						%Recovery
	Before addition			After addition			
	Predicted	Observed	%RSD	Calculated	Observed	%RSD	
Reference Standard	16	15.83 (0.12)	0.77	-	-	-	98.94
	20	19.98 (0.08)	0.39	-	-	-	99.90
	24	23.90 (0.36)	1.52	-	-	-	99.58
Standard Addition to Sample	16	16.86 (0.17)	0.98	20.06	20.32 (0.32)	1.56	101.30
	20	20.78 (0.19)	0.94	23.83	24.04 (0.17)	0.73	100.88
	24	25.51 (0.10)	0.37	28.38	28.44 (0.10)	0.34	100.21

Abbreviations: SD: - standard deviation; RSD: - relative standard deviation

Table 8: Summary of the optical characteristics and validation parameters

Parameters	Results
Detection wavelength (λ_{max})	276 nm
Linearity range	5 - 45 µg/mL
Regression equation	$y = 0.0264x + 0.0058$
Correlation coefficient (r^2)	0.9999
Limit of detection	0.3886 µg/mL
Limit of quantification	1.1775 µg/mL
Molar absorptivity	8,952 ± 113 $L.mol^{-1}.cm^{-1}$
Sandell's sensitivity	0.0373 $\mu g.cm^{-2}$
Precision (%RSD)	
Repeatability	0.72 - 1.54
Robustness	0.33 - 0.86
Intermediate precision	0.72 - 1.33
Ruggedness	0.48 - 1.54
Accuracy (%Recovery)	98.9 - 101.3

Abbreviations: RSD: - relative standard deviation; λmax: - wavelength of maximum absorption

Assay of diclofenac in tablet formulation

Assay result of the four studied brands is presented in Table 9. The average %contents of diclofenac in tablet formulation of the two representative batches of each brand were approximately 45% (DCF-A), 98% (DCF-B), 104% (DCF-C) and 105% (DCF-D). The %RSD for all the determinations were all < 2.

Table 9 : Assay of selected brands for the diclofenac potassium content of tablets

Sample code	Batch	Amount found (SD)	%RSD	%Content	Average %content
DCF-A	5014	49.85 (0.66)	1.32	49.85	44.61
	5015	39.37 (0.55)	1.39	39.37	
DCF-B	7069	91.52 (0.57)	0.62	91.52	97.71
	7070	103.89 (0.97)	0.94	103.89	
DCF-C	04	100.23 (1.05)	1.05	100.23	103.45
	17	106.67 (1.33)	1.24	106.67	
DCF-D	513	104.90 (0.85)	0.81	104.90	105.03
	889	105.15 (1.24)	1.18	105.15	

Abbreviations: SD: - standard deviation; RSD: - relative standard deviation

DISCUSSION

Diclofenac is one of the therapeutically important medicines on the National Essential Medicines List[30]. Based on the British Pharmacopoeia potency specification of 95 – 105% for diclofenac tablet[24], DCF-B, DCF-C and DCF-D passed while DCF-A with an average content of 45% failed the potency test. Validation results of the assay method demonstrated good repeatability and intermediate precision with < 2 %RSD for all the determinations as presented in Table 8.

Result of the accuracy test of spiked samples also indicated that the method results were unaffected by excipients of the formulation. Comparison of the present study result with the two previous Nigerian studies which also evaluated %content of the active ingredient of different brands of diclofenac tablets showed that this is the only study to report poor-quality diclofenac tablets as a result of decreased % content of the active ingredient. Ayorinde *et al.* (2012) found all the five investigated brands to be of good quality. However, Kirim *et al.* (2014) reported three out of the seven studied brands to be of poor quality due to their higher % contents, above the upper limit of the BP specification of 95 – 105% [18,19]. The present study found one of the four studied brands to be falsified, due to the misrepresentation of its composition (with < 50% active diclofenac content of the labelled claim). The observed potency value is far below the lower limit of the BP specification in spite of having the required regulatory agency's approval as well as passing the other pharmaceutical quality tests. The falsified brand had India enlisted as its country of manufacture with a longer shelf life of 4 years as against the average of 2/3 years observed for other brands. Asia accounts for the biggest share of the trade in substandard and falsified medicines, according to the industry-funded organization, the Pharmaceutical Security Institute[31,32]. The World Health Organization (WHO) estimates that

approximately 30% of the medicines sold in parts of Asia, Africa, and Latin America are substandard and falsified[31]. It is also generally believed that pharmaceuticals meant for export are not strictly regulated by exporting countries to the same standards as those produced for their domestic consumption[21]. Use of poor-quality analgesics are detrimental to health, and the clinical implications may include, increased likelihood of therapy failure, exacerbation of the conditions being treated, abuse of NSAIDs, overdose, and increased cost of medical treatment. Lack of appropriate legislation and weak regulatory enforcement of the existing legislation controlling the manufacture, importation, distribution, supply and sales of drugs are among the factors militating against the efforts at combating substandard and falsified medicines in the country. Equally of concern is the problem of weak penal sanctions for violators of the existing drugs legislation. Thus, the development of a more effective national drug regulatory system, as well as the strengthening of the national legislative frameworks are required to fight this menace of poor-quality medicines currently ravaging the country. In addition, the National Agency for Food and Drug Administration and Control (NAFDAC) must increase surveillance to ensure that quality of, not only the locally manufactured but also imported drugs, are periodically assessed and monitored.

Although the selection of only four out of the numerous brands of diclofenac marketed in the country was randomly carried out, the sample size may not be sufficient to provide adequate representation, and the sampling technique may be viewed as more of convenient rather than random sampling. Additionally, dissolution is considered as one of the most important quality control tests. However, dissolution characters of the brands were not determined due to lack of access to the dissolution apparatus. Nevertheless, the result of this pilot study is sufficient to provide the initial signal of the problem of falsification of this product by manufacturers.

CONCLUSION

In summary, this study detected a substandard and falsified brand out of the four tested brands of diclofenac tablets. This provides a clue about the prevalence of poor-quality medicines in the locality and the country at large. More research on quality assessment of medicines from other therapeutic categories is warranted, in particular, drugs used in the treatment of life-threatening diseases.

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CONFLICT OF INTEREST

All the authors declare that there are no conflicts of interest.

REFERENCES

1) Funestrand H1, Liu R, Lundin S and Troein M (2019). Substandard and falsified medical products are a global public health threat. A pilot survey of awareness among physicians in Sweden. Journal of Public Health (Oxf) 41(1): e95-e102. https://doi.org/10.1093/pubmed/fdy092.
2) World Health Organization (2017). WHO Global Surveillance and Monitoring System for substandard and falsified medical products. Geneva: World Health Organization. https://apps.who.int/iris/bitstream/handle/10665/326708/9789241513425-eng.pdf?ua=1.
3) World Health Organization (2017). A study on the public health and socioeconomic impact of substandard and falsified medical products. https://www.who.int/medicines/regulation/ssffc/publications/Layout-SEstudy-WEB.pdf?ua=1.
4) Altman R, Bosch B, Brune K, Patrignani P and Young C (2015). Advances in NSAID development: evolution of diclofenac products using pharmaceutical technology. Drugs 75(8): 859-877.
5) McGettigan P and Henry D (2013). Use of nonsteroidal anti-inflammatory drugs

that elevate cardiovascular risk: an examination of sales and essential medicines lists in low-, middle-, and high-income countries. PLOS Medicine 10(2): e1001388.

6) Todd PA and Sorkin EM (1988). Diclofenac sodium. A reappraisal of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. Drugs 35(3): 244-285.
7) Chuasuwan B, Binjesoh V, Polli JE, Zhang H, Amidon GL, Junginger HE, Midha KK, Shah VP, Stavchansky S, Dressman JB and Barends DM (2009). Biowaiver monographs for immediate release solid oral dosage forms: diclofenac sodium and diclofenac potassium. Journal of Pharmaceutical Sciences 98(4):1206-1219.
8) Cryer B and Feldman M (1998). Cyclooxygenase-1 and cyclooxygenase-2 selectivity of widely used nonsteroidal anti-inflammatory drugs. American Journal of Medicine 104(5) :413-421.
9) Sallmann AR (1986). The history of diclofenac. American Journal of Medicine 80(4B):29-33.
10) Ku EC, Lee W, Kothari HV, Kimble EF, Liauw L and Tjan J (1985). The effects of diclofenac sodium on arachidonic acid metabolism. Seminars in Arthritis and Rheumatism 15(2 Suppl 1): 36-41.
11) Ku EC, Lee W, Kothari HV and Scholer DW (1986). Effect of diclofenac sodium on the arachidonic acid cascade. American Journal of Medicine 80(4B): 18-23.
12) Takayama K, Hirose A, Suda I, Miyazaki A, Oguchi M, Onotogi M and Fotopoulos G (2011). Comparison of the anti-inflammatory and analgesic effects in rats of diclofenac-sodium, felbinac and indomethacin patches. International Journal of Biomedical Science 7(3): 222-229.
13) Pantziarka P, Sukhatme V, Bouche G, Meheus L and Sukhatme VP (2016). Repurposing Drugs in Oncology (ReDO)-diclofenac as an anti-cancer agent. Ecancermedicalscience 10:610. https://doi.org/10.3332/ecancer.2016.610.
14) Kelesidis T and Falagas ME (2015). Substandard/counterfeit antimicrobial drugs. Clinical Microbiology Reviews 28(2): 443-464.
15) Pincock S (2003). WHO tries to tackle problem of counterfeit medicines in Asia. British Medical Journal 327(7424): 1126. https://doi.org/10.1136/bmj.327.7424.1126-a.
16) GBD 2017 Disease and Injury Incidence and Prevalence Collaborators (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet (London, England). 392(10159): 1789–1858.
17) Khaskheli AR, Abro SK, Sherazi STH, Afridi HI, Mahesar SA and Saeed M (2009). Simpler and Faster Spectrophotometric Determination of Diclofenac Sodium in Tablets, Serum and Urine Samples. Pakistan Journal of Analytical and Environmental Chemistry 10(1 & 2): 53-58.
18) Ayorinde JO, Odeniyi MA and Itiola AO (2012). Evaluation of pharmaceutical and chemical equivalence of selected brands of diclofenac sodium tablets. East and Central African Journal of Pharmaceutical Sciences 15: 3-9.
19) Kirim RA, Mustapha KB, Isimi CY Ache T, Sadiq A, Galadima IH and Gamaniel KS (2014). Quality assessment of different brands of diclofenac tablets in some pharmacy stores in Abuja. African Journal of Pharmacy and Pharmacology 8(37): 924-928.
20) Newton PN, Lee SJ,

Goodman C, Fernández FM, Yeung S, Phanouvong S, Kaur H, Amin AA, Whitty CJ, Kokwaro GO, Lindegårdh N, Lukulay P, White LJ, Day NP, Green MD and White NJ (2009). Guidelines for field surveys of the quality of medicines: a proposal. PLOS Medicine 6(3): e52. https://doi.org/10.1371/journal.pmed.1000052

21) World Health Organization (1999). WHO counterfeit drugs. Guidelines for the development of measures to combat counterfeit drugs, vol 99.1. World Health Organization, Geneva. http://www.who.int/medicines/publications/counterfeitguidelines/en/.
22) World Health Organization (2008). The International Pharmacopoeia. Geneva: World Health Organization, Dept. of Essential Medicines and Pharmaceutical Policies.
23) Mathur N, Kumar R, Tiwari K, Singh S and Fatima N (2015). Evaluation of quality control parameters on various brands of paracetamol tablet formulation. World Journal of Pharmaceutical Sciences 4(7): 976-984.
24) British Pharmacopoeia (2004). Diclofenac preparations. The Stationery Office. Vol. III. 2354-2355, London.
25) The United States Pharmacopeia and National Formulary (2007). U.S. Pharmacopeia National Formulary USP 30 NF 25, the United States Pharmacopeia, Inc. 276; 2327.
26) ICH (2005). ICH Harmonized Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology, Q2(R1), International Conference on Harmonization, Geneva, Switzerland. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf.
27) Antignac M, Diop BI, Do B, N'Guetta R, Toure IA, Zabsonre P and Jouven X (2017). Quality assessment of 7 cardiovascular drugs in 10 sub-Saharan countries: the SEVEN study. JAMA Cardiology 2(2): 223–225.
28) Dhokale KK, Deore DD and Nagras MA (2016). UV spectrophotometric method for simultaneous estimation of diclofenac salt and eperisone hydrochloride in bulk and capsule dosage form. International Journal of Pharmaceutical Sciences and Research 7(9):3810 – 3814.
29) Gunji R, Nadendla RR and Ponnuru VS (2012). Simultaneous UV-spectrophotometric determination and validation of diclofenac sodium and rabeprazole sodium using hydrotropic agents in its tablet dosage form. International Journal of Drug Development and Research 4(1): 316-324.
30) Federal Ministry of Health/World Health Organization (2016). Federal Republic of Nigeria Essential Medicines List, sixth edition. https://www.medbox.org/document/nigeria-essential-medicines-list
31) World Health Organization (2010). Growing threat from counterfeit medicines. Bulletin of the World Health Organization 88(4):247-248. https://www.who.int/bulletin/volumes/88/4/10-020410.pdf?ua=1.
32) Pharmaceutical Security Institute (2018). Counterfeit situation: Arrest data. https://www.psi-inc.org/arrest-data.