ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

FIXED SIZED SIMPLEX OPTIMIZATION OF SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATIVE DETERMINATION OF DICLOFENAC IN PHARMACEUTICAL PREPARATIONS

A. A. NUHU, M. S. SALLAU, TUKUR BALA*
Department of Chemistry, Ahmadu Bello University, Zaria.

Manuscript Info

Manuscript History:

Received: 25 August 2015
Final Accepted: 26 September 2015
Published Online: October 2015

Key words:

Method development, pharmaceutical preparation, brown complex, simplex optimization, diclofenac sodium.

Corresponding Author

TUKUR BALA

Abstract

A simple method was developed for spectrophotometric determination of diclofenac sodium in pharmaceutical preparations using potassium permanganate to form a brown complex in the presence of slightly acidic sulphuric acid media. This complex was monitored at 455nm wavelength. Simplex optimization procedure was used to optimize the factor levels, and the method was validated and successfully applied to the assay of 11 brands of the pharmaceutically-formulated analyte. Calibration curve was linear within the range of 5µg/L – 35µg/L concentrations (r^2= 0.998). The method was precise as shown by the intra-day (repeatability) and inter-day (reproducibility) indices of precision, 0.56% and 0.45% respectively. The limit of detection was estimated to be 0.158mg/L, while the limit of quantitation was 0.525mg/L. Recoveries were calculated as 89.78% - 104%. Statistical paired t–test showed that there was no significant difference on comparing the developed method with the official method in British Pharmacopeia. However, Chauvenet's criterion applied to the data revealed that "SortenForte" was an outlier and therefore a possible counterfeit or substandard formulation. The newly developed method compared favorably, and can effectively serve as an alternative, to the official method for routine determination of the analyte in pharmaceutical preparations.

INTRODUCTION

Anti-inflammatory and analgesic properties of non-steroidal anti-inflammatory drugs (NSAIDs) are widely exploited in the management of musculoskeletal diseases. These drugs are applied both for acute and long-term chronic cases (Gabriel and Matteson, 1995; Zochling *et al*., 2006; Hochberg, 2005). The most common type of arthritis is osteoarthritis (osteoarthrosis), which is a degenerative joint disease. It is a common cause of chronic debilitation in adults (Brooks, 2002), and its prevalence varies among populations, but increases globally with age. One fifth of adults beyond the age of 65 years are estimated to be affected by knee osteoarthritis. Some of these patients may be asymptomatic, and the condition quietly progresses to disability (Hochberg, 2001; Dieppe *et al*., 1997).

Oral NSAIDs have potential toxicities and can limit their use and must, therefore, be monitored, especially in aged persons (Brooks, 2002). Prescription may be given as single dose or in short-term alternating therapy, but 21 days may be required in order to have an appreciable level of the anti-inflammatory effect. Some NSAIDs are also useful in the management of postoperative pain (Biudat-Russel and Gabriel, 2001).

Diclofenac sodium [Sodium (o- {(2, 6-dichlorophenyl) amino} phenyl) acetate] (Figure 1) is a synthetic non-steroidal anti-inflammatory drug which has been very effective in the management of various forms of inflammatory and rheumatoid conditions (USP, 2002).

Diclofenac sodium occurs as a white to slightly yellowish, slightly hygroscopic, crystalline powder (BP, 2013). It is sparingly soluble in water and alcohol; slightly soluble in acetone; freely soluble in methyl alcohol, and insoluble in chloroform (Bahram, 2008). A number of analytical methods have been developed for the quantitative determination of this drug in dosage forms and in biological samples. These methods utilize electrochemical techniques such as cyclic voltammetry (Blanco-Lopez *et al.*, 2004), potentiometric sensor (Mojtaba *et al*., 2005), and capillary electrophoresis (Solangi *et al*., 2009). However, the official method in the United States Pharmacopoeia (USP) is by liquid chromatographic determination (USP, 2002; Arcelloni *et al*., 2001).

Monzon *et al.* (2012) has introduced a kinetic spectrophotometric method for quantification of diclofenac in pharmaceutical preparations. The redox reaction rate between diclofenac and potassium permanganate ($KMnO_4$) in acidic environment was determined and good linearity was obtained. The reaction of diclofenac and $KMnO_4$ in weakly acidic media was earlier employed by Sultan *et al.* (2010) in the determination of this analyte using a sequential injection analysis. One-variable-at-a-time optimization approach was followed in that determination. We report in this work a simple, cost effective and solvent minimization approach for the determination of diclofenac in pharmaceutical preparations using the reaction of the analyte with $KMnO_4$ in mildly acidic media, and the method was optimized using simplex optimization procedure.

MATERIALS AND METHODS

Materials

Single beam UV/Vis spectrophotometer (Janway 6405), analytical weighing balance (Satorious ED224S), and potentiometer were used. Analytical grade diclofenac sodium standard was supplied by SPIMACO (Saudi Arabia) and pharmaceutically prepared diclofenac sodium formulations were obtained from different manufacturers (Table 1).

Other materials used are: Beakers, Conical flask, Syringe, Micro pipette, Burette, Mortar and pestle.

Chemicals

All chemicals used were of analytical grade and they include: Potassium permanganate (Wilkson vickers LTD 0548), sulphuric acid (Sigma Aldrich, lot 83180), and glacial acetic acid (Loba Chemie Pvt limited, lot LMO5971311), acetic acid, and Perchloric acid (Loba chemie pvt LTD).

Preparation of Solutions and Reagents

All reagents were prepared according to standard method of reagent preparations (Ojokuku, 2012) using deionized water.

Preparation of stock solutions and working standard solutions of diclofenac sodium

Standard diclofenac sodium was freshly prepared by dissolving 4g in deionized water to give concentration of 4000mg/L (4 mg/mL).Working standard solutions of diclofenac sodium were freshly prepared at the concentrations of 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8 and 2 mg/mL.

Preparation of stock solution and working solutions of potassium permanganate

A stock solution of potassium permanganate was freshly prepared by dissolving 1.58g in 1L volumetric flask. From the stock solution, working solutions of 0.079, 0.158, 0.237, 0.316, 0.395, 0.474, 0.553, 0.632, 0.711, 0.79, 0.869, 0.948, 1.027, 1.106, 1.185, and 1.264 mg/mL were used.

Preparation of stock solution and working solutions of sulphuric acid

A stock solution of 0.01M sulphuric acid was prepared by diluting 0.53mL concentrated sulphuric acid in 1L volumetric flask and, from the stock, a concentration of $5x10^{-6}$M was further prepared. Working solutions of $2.5x10^{-9}$, $5x10^{-9}$, $7.5x10^{-9}$, $1x10^{-8}$, $1.25x10^{-8}$, $1.5x10^{-8}$, $1.75x10^{-8}$ and $2x10^{-8}$M were used.

Preparation of 0.1M Perchloric acid

Exactly 8.5mL of Perchloric acid was placed in a volumetric flask containing about 900 mL of glacial acetic acid and mixed. Acetic anhydride (30 mL) was added and this was diluted to 1L with glacial acetic acid; it was mixed and allowed to stand for 24 hours.

Preparation of sample solution of diclofenac sodium

The average weights of diclofenac sodium (pharmaceutical preparations) were determined by weighing 10 tablets and these were then ground into powdered form in a mortar. Amounts equivalent to 20mg/L diclofenac were used for spectrophotometric determination.

Method Development

The method was developed by reacting diclofenac sodium and potassium permanganate in presence of mild sulphuric acid concentration. The reaction leads to formation of brown complex through a redox reaction. Wavelength scanning was performed in order to differentiate the wavelength for the absorbance of potassium permanganate from that of complex and these were carried out in the range of 450 – 700 nm. Effect of time on the reaction was monitored by taking the absorbance readings of the complex from time zero until the reading became stable.

Optimization Procedure

In this work, three factors were optimized, two at a time. Three sets of optimizations were carried out from the three factors: diclofenac-sulphuric acid, sulphuric acid-potassium permanganate and potassium permanganate-diclofenac sodium sets of optimization. The set that gave the best responses was chosen for the optimized factor levels. We measured the response for each set of factor levels and named them as best (Vb) for the highest response value, second best (Vs) for the second highest response value and worst (Vw) for the least response value, replacing the (Vw) with new set of factor levels called new vertex (Vn) using a set of simplex rules. This process continued until a global optimum was reached or until no further optimum was possible. The starting factor levels were 4 mg/mL diclofenac sodium, 5 x 10^{-9} M sulphuric acid and 0.632 mg/mL $KMnO_4$.Throughout this optimization procedure, a fixed step size of 0.5 was maintained for both factors in each simplex (Spendley *et al*., 1962; Deming and Parker, 1978).

Method Validation

The method was validated using linearity, precision, limit of detection (LOD), limit of quantitation (LOQ), and accuracy (recovery) in accordance with ICH guideline (Walfish, 2006; Prashanthi *et al*., 2012).

Linearity and calibration curve

The linearity was determined from calibration curve data through coefficient of determination. The calibration curve was constructed in the concentration range of 5µg/mL to 35µg/mL.

Precision

Intra-day precision was determined by performing triplicate experiments in a day at two different concentration levels. For inter-day precision, however, samples were prepared in triplicates for five days to determine the reproducibility, and the results were expressed as percent relative standard deviation (%RSD). From the calibration data, standard concentration of 10mg/L and 30mg/L were chosen for carrying out both the inter-day (reproducibility) and intra-day (repeatability) precision calculations. The initial concentrations of diclofenac sodium, sulphuric acid and potassium permanganate were 500mg/L, 5X10^{-9} Mand1.58mg/mL respectively.

Limit of detection (LOD) and limit quantification (LOQ)

LOD and LOQ were estimated using equations 1 and 2respectively.

LOD = 3 X S / M……Equation 1

LOQ = 10 X S / M……..Equation 2

Where S = noise of the estimate or the standard deviation of the determination and M is the slope of the calibration graph.

Recovery (Accuracy)

An equivalent amount of 20mg/L of diclofenac sodium and the optimized factor levels of potassium permanganate was allowed standing for 20 minutes for the reaction to complete and spectrophotometric analysis was carried out at a wavelength of 455nm.

Potentiometric Determination of Diclofenac Sodium

Exactly 250mg of each of the eleven brands of diclofenac sodium tablets used were dissolved in 30mL of glacial acetic acid and titrated with 0.1M Perchloric acid; the end point was determined potentiometrically, one 1ml of 0.1 M Perchloric acid used is equivalent to 31.81 mg of diclofenac sodium (BP, 2013).

Statistical Analyses

Paired t-test was used to compare simplex optimization procedure with official method to ascertain whether significant difference existed between the developed method and the official method (BP, 2013).

RESULTS AND DISCUSSION

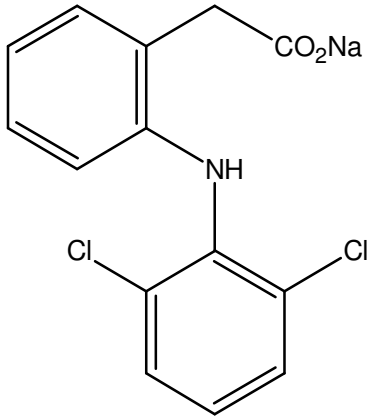

Figure 1: Chemical structure of diclofenac sodium

Table 1: Composition of pharmaceutically-prepared diclofenac sodium tablets

Trade name	Weight of tablet (g)	Composition	Mg
Dicloktis-plus	0.95109	Diclofenac sodium	50
		Paracetamol	500
		Excipient	Q.S
Sureclofen Forte	1.08	Diclofenac sodium	100
		Excipient	Q.S
Dicnac 550	0.75155	Diclofenac sodium	50
		Paracetamol	500
Sorfen Forte	0.82788	Diclofenac sodium	50
BaterenDexcel	0.21840	Diclofenac sodium	50
Dolo Meta B	0.64078	Diclofenac sodium	50
		Vitamin B1	50
		Vitamin B6	100
		Vitamin B12	100
Voltaren	0.30108	Diclofenac sodium	100
Clofenac	0.21373	Diclofenac sodium	50
Olfen	0.24838	Diclofenac sodium	50
Arthrotec	0.50030	Diclofenac sodium	75
Lofnac 100	0.38243	Diclofenac sodium	50

Effect of Time on Reaction

The time it took for the reaction to complete was studied from time 0 minute until it became stable at 20 minute. Previous findings have put this value at the minimum of 15 minutes (Younes, 2014; Kumar *et al*., 2012).

Reaction Mechanism

The reaction is based on redox reaction between diclofenac sodium and potassium permanganate in the presence of slightly acidic media leading to the formation of a brown colored complex that was detected at 455 nm after 20 min. At higher concentration of sulphuric acid, the diclofenac would become precipitated and will affect absorbance (Monzon *et al*., 2012), while concentration below the optimized level of acid leads to very slow reaction.
Amines are excellent electron donors and can strongly interact with electron acceptors; diclofenac which has secondary amine group can act as electron donor and can react with potassium permanganate as shown in (Figure 2). Using electron spin resonance scan, Sultan *et al.* (2010) have found a stable sixtet hyperfine splitting which was previously attributed to manganese (II) (Morsy and Khaled, 2002). These earlier researchers also found a weak triplet of sixtet hyperfine splitting which was believed to correspond to the quinone imide form of the drug (Vanderford and Snyder, 2006); a super imposed radical feature peak was obtained and ascribed to the oxidized drug in a diradical species (Sultan *et al*., 2010). The reaction mechanism was, therefore, proposed in two steps. First step involves the formation of an intermediate dication radical. This is followed by the second step that involves the formation of stable brown colored product, the oxidized brown colored complex of the quinone imide form of the drug (Sultan *et al*., 2010).

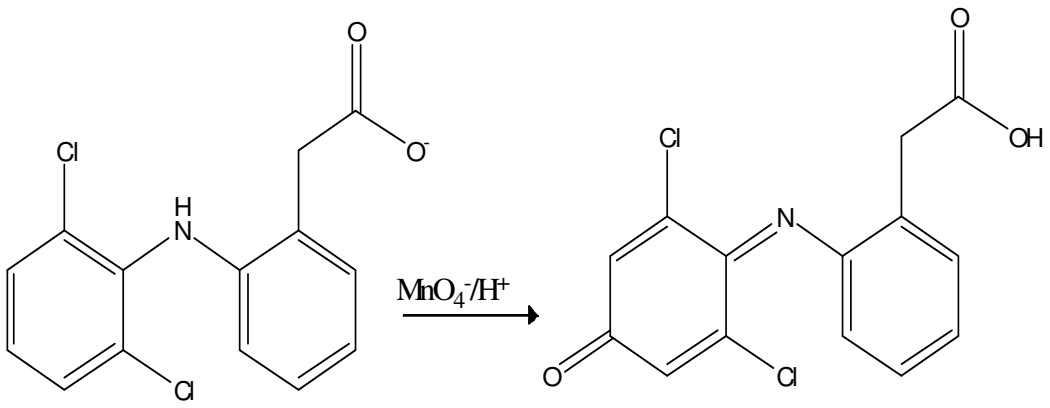

Figure 2: Proposed reaction scheme for the oxidization of diclofenac sodium with potassium permanganate in acidic media (adopted from Sultan *et al*., 2010).

Absorbance Vs Wavelength Profile for Potassium Permanganate

The absorbance of potassium permanganate from 450nm to 700 nm shows a maximum at 515 nm (Figure 3). This agrees with what was reported by adeeyinwo *et al.* (2013).

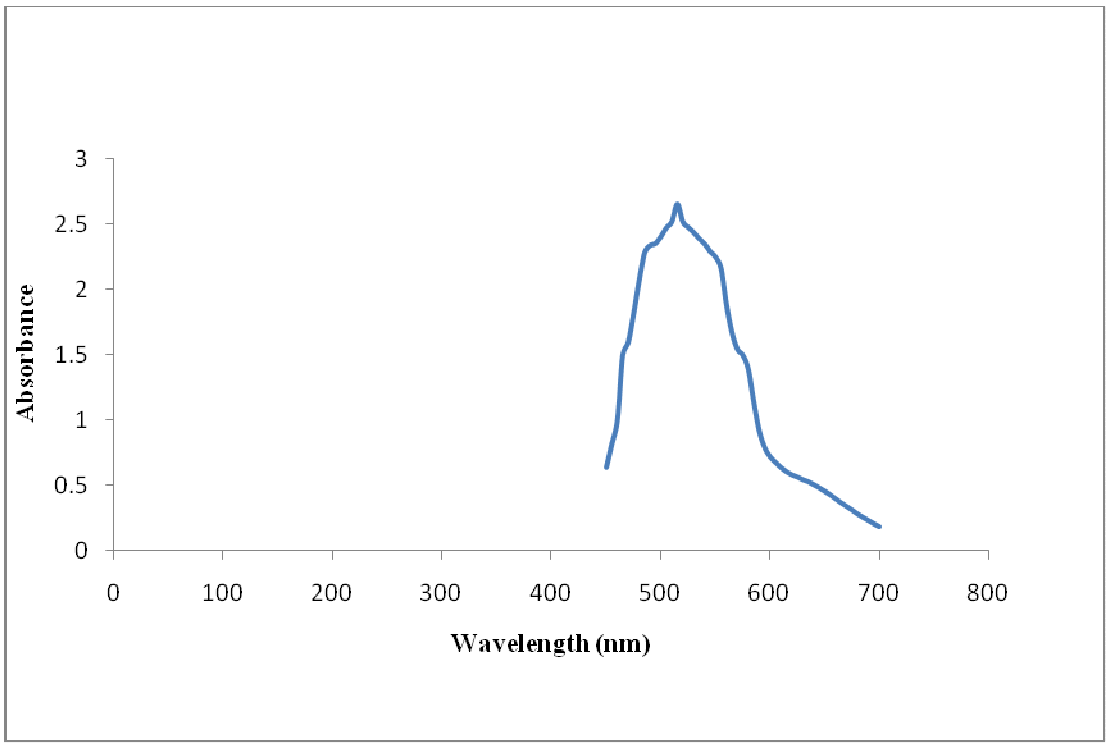

Figure 2: Absorbance scan for potassium permanganate.

Simplex Optimization

For simplex optimization sets (Figures 4 – 6) the optimized factor levels (Table 2) were found to be 0.632mg/L (potassium permanganate),1.1X10^{-9}M (sulphuric acid) and 0.72mg/L (diclofenac sodium), and these agreed with what was reported by Idris (2011).

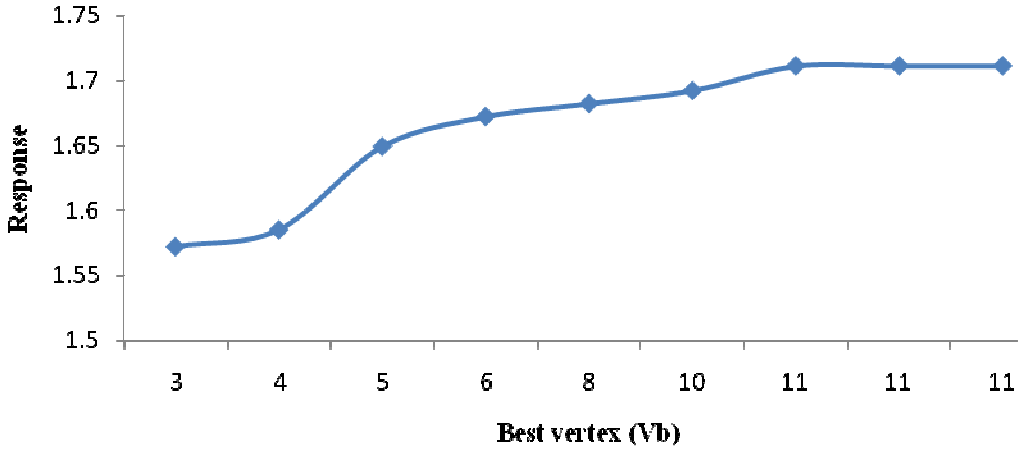

Figure 4: Simplex optimization profile for diclofenac sodium – sulphuric acid set

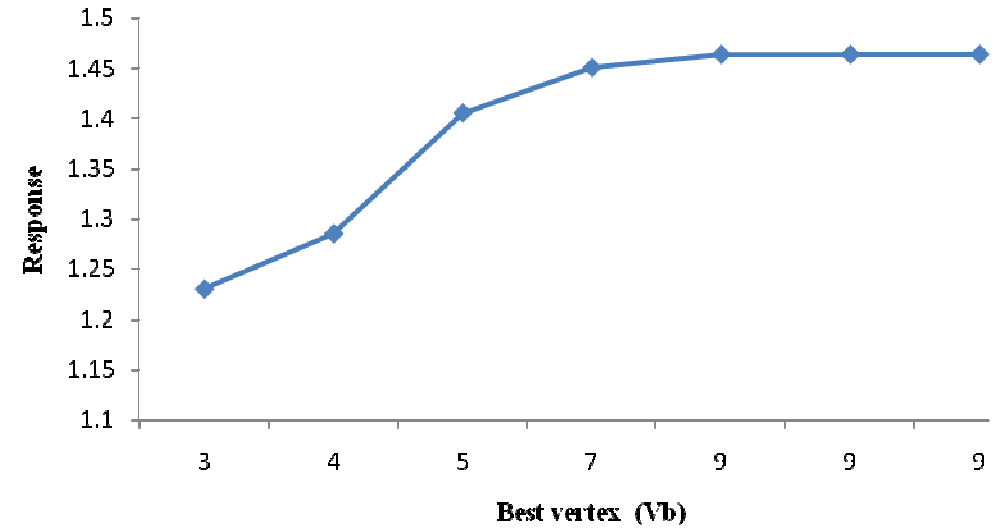

Figure 5: Simplex optimization profile for sulphuric acid – potassium permanganate set

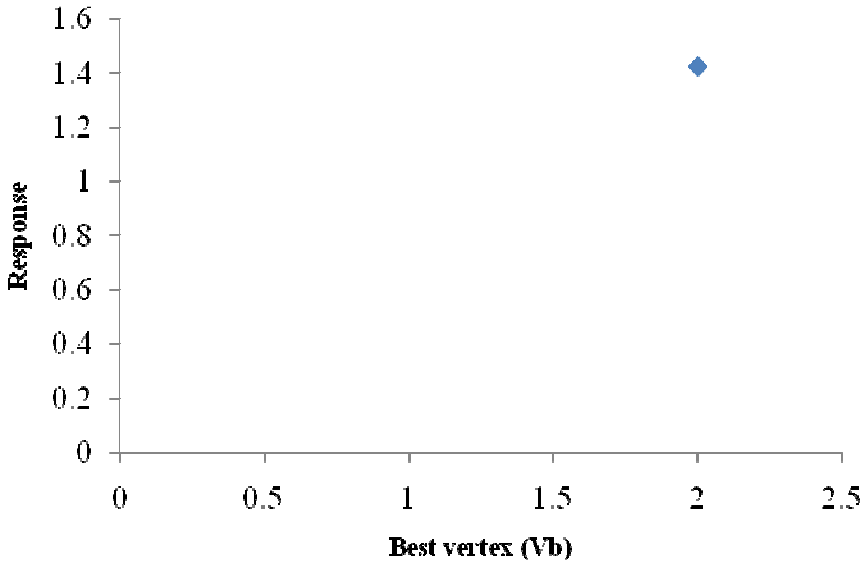

Figure 6: Simplex optimization profile for potassium permanganate – diclofenac sodium set

Table 2: Simplex Optimized Factor Levels

Factor	Factor level
Potassium permanganate	0.632mg/mL
Sulphuric acid	$1.1X10^{-5}M$
Diclofenac sodium	0.72mg/mL

Method Validation

The developed method was found to be linear as expressed by the coefficient of determination, $r^2 = 0.998$ (Figure 7) and this agreed with what was reported by Khaskheli *et al.* (2009). The method was found to be precise; repeatability and reproducibility have % RSD of 0.56% and 0.45% respectively (Tables 3 and 4) and these agreed with what was reported by Alagar *et al.* (2013). The LOD and LOQ were estimated to be 0.158mg/L and 0.525mg/L which agreed with the findings of Beeravolu *et al.* (2012). Recovery (%) was found to be within the range of 89.78% - 104% (Table 5) and this agreed with what was reported by Ebeshi *et al.* (2014) and indicates that excipients did not have significant interference on the accuracy of determination.

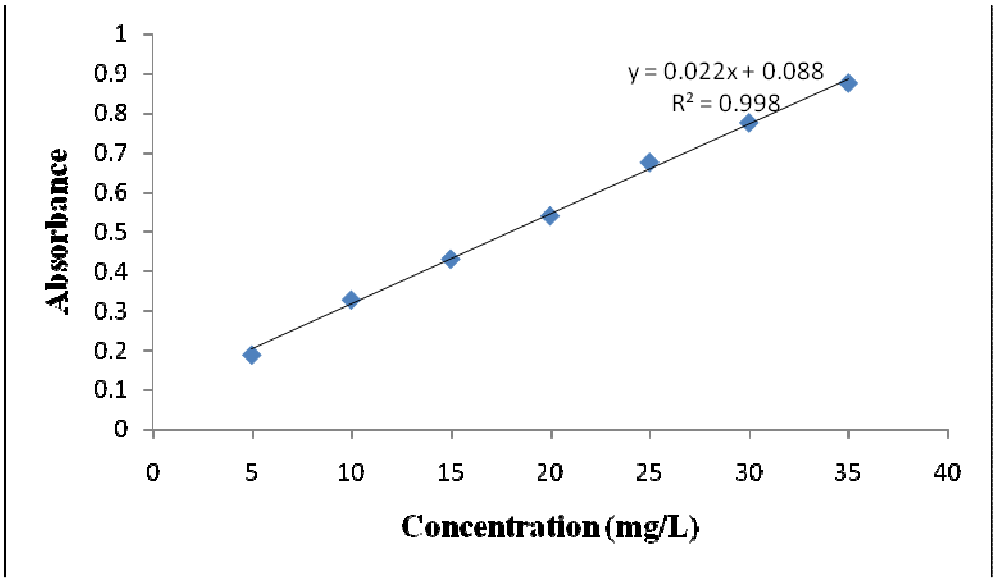

Figure7: Calibration curve for simplex optimization

Table 3: Intra-day precision (Repeatability)

Diclofenac, mg/L	Mean response	Standard deviation	%RSD
10	0.327	$3.65X10^{-3}$	1.12
30	0.772	$5.0X10^{-6}$	$6.47X10^{-4}$
Average			0.56

Table 4: Inter-day precision (reproducibility)

Diclofenac, mg/L	Mean response	Standard deviation	% RSD
10	0.329	0.0022	0.66
30	0.773	0.0019	0.24
Average			0.45

Table 5: Recovery result for simplex optimization

Sample	Concentration used (mg/L)	Amount reported (mg)	Amount found (mg)	% Recovery
Dicloktis-plus	20	50	44.89	89.78
SureclofenForte	20	100	92.61	92.61
Dicnac 550	20	50	45.76	91.52
Sorfen Forte	20	50	13.26	26.52
BaterenDexcel	20	50	50.2	100.44

Dolo Meta B	20	50	48.37	96.74
Voltaren	20	100	103.04	103.04
Clofenac	20	50	44.78	89.57
Olfen	20	50	49.13	98.26
Arthrotec	20	75	77.77	103.67
Lofnac 100	20	50	52	104

Recovery (Accuracy) by Official Method

The recovery by official method was found to be within the range of 84.09 – 107.95 (Table 6). This agreed with what was reported by Ebeshi *et al.* (2014).

Table 6: Accuracy (Recovery) Using Official Method

Sample	Concentration used (mg/L)	Amount reported (mg)	Amount (mg)	yield	% Recovery[a]
Dicloktis-plus	20	50	43.37		86.74
SureclofenForte	20	100	97.35		97.35
Dicnac 550	20	50	42.05		84.09
Sorfen forte	20	50	10.24		20.47
BaterenDexcel	20	50	52.65		105.3
Dolo Meta B	20	50	47.35		94.7
Voltaren	20	100	92.04		92.04
Clofenac	20	50	48.67		97.34
Olfen	20	50	43.37		86.74
Arthrotec	20	75	78.97		105.29
Lofnac 100	20	50	53.97		107.95

[a] BP, 2013

Statistical Analysis

From the statistical analysis, paired t–test shows that there was no significant difference between simplex optimization and official method reported in British Pharmacopeia as shown in Table 7. This is because t-statistics was less than t critical. However, Chauvenet's criterion (Table 8) applied to the procedures reveal that "Sorten Forte" was an outlier because $d_{max} > \tau_{max} X\ S_X$, and therefore, a possible counterfeit or sub-standard formulation (Nuhu, 2011).

Table 7: Comparison between Simplex Optimization and Official (BP) Method

	Variable 1	*Variable 2*
Mean	90.56045455	88.90872727
Variance	481.1669615	580.1493278
Observations	11	11
Pearson Correlation	0.961432306	
Hypothesized Mean Difference	0	
Df	10	
t Stat	0.813210114	
P(T<=t) one-tail	0.217515946	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.435031892	
t Critical two-tail	2.228138842	

Variable 1: Simplex optimization procedure, Variable 2: Official method

Table 8: Chauvenet's Criterion for Simplex Optimization Procedure

Sample	% Recovery	Mean	d_{max}	S_x	τ_{max}
Sorfen forte	26.52	90.56	64.05	21.93	2
Clofenac	89.57				
Dicloktis-plus	89.78				
Dicnac 550	91.52				
Sureclofen	92.61				
Dolo Meta B	96.74				
Olfen	98.26				
BaterenDexcel	100.44				
Voltaren	103.04				
Arthrotec	103.67				
Lofnac 100	104				

CONCLUSIONS

A novel, simple, cost and time effective spectrophotometric analytical method for the determination of diclofenac sodium in pharmaceutical preparations was successfully developed. Statistical analysis showed that there was no significant difference between the accuracy of the developed method and official BP method. But the developed method has several advantages over the official method. These include shorter time of analysis and lesser total reaction cell volume. Therefore, the developed method could effectively serve as an alternative to the contemporary method for routine determination of the analyte in pharmaceutical formulations.

ACKNOWLEDGMENT

The authors acknowledge Professor Salah M. Sultan for the gift of diclofenac sodium standard, and author TB appreciates ABU Zaria for providing the platform for this research leading to his M.Sc. degree.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this article.

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