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One – Factor – At –A- Time Optimization of Spectrophotometric Method for the Determination of Diclofenac in Pharmaceutical Preparations

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ABSTRACT

One – factor – at – a – time optimization procedure (OFAT) was developed for spectrophotometric determination of diclofenac sodium in pharmaceutical preparations using potassium permanganate to form complex compound in the presence of slightly acidic medium and the complex form of the drug was monitored at a wavelength of 455nm. The method was validated and successfully applied to the assay of 11 brands of the pharmaceutically formulated drugs. Calibration curve was linear within the range of 5µg/L - 35µg/L with correlation coefficient (r^2) of 0.994 respectively. The procedure was precise, as determined by %RSDof 0.3% and 0.1% for intra-day (repeatability) and inter day precision (reproducibility) respectively. Limit of detection and limit of quantification was found to be 0.13mg/L and 0.43mg/L respectively. Recoveries were obtained as 85.86 – 110.53%. The statistical paired t – test showed that there was no significant difference comparing the developed method with the official (BP) method. Chauvenet's criterion result 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 alternative to the contemporary method for routine determination of the analyte in pharmaceutical formulations.

Keywords: *Method development, pharmaceutical formulation, analysis, optimization, diclofenac*

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are chemically heterogeneous group of compounds that share certain therapeutic actions and adverse effects[1]. They are commonly prescribed medications for the treatment of musculoskeletal disorders. Osteoarthritis is the most common form of arthritis in humans and its prevalence rises with age[2]. Unfortunately, however, oral NSAIDs have potential toxicities that must be monitored for and can limit the use of these drugs in certain populations including people of older age[3]. It may be given as single dose or in short-term alternating therapy. The anti-inflammatory effects become apparent when it is taken for up to 21 days. Combined anti-inflammatory and analgesic effects make them helpful for the symptomatic relief of painful situations. Some NSAIDs are used in the management of postoperative pain[4].

Diclofenac sodium [Sodium (o- {(2, 6-dichlorophenyl) amino} phenyl) acetate] (Figure 1) is a synthetic non-steroidal anti-inflammatory drug (NSAID) which has been proven as a safe and efficacious drug in the treatment of a variety of inflammatory and rheumatoid disorders[5].

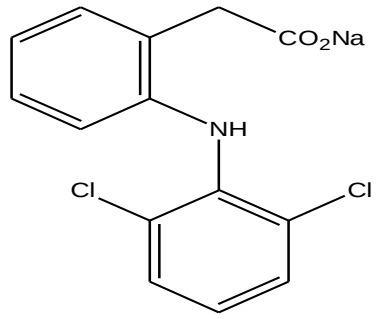

Figure 1: Chemical Structure of Diclofenac Sodium

According to British Pharmacopoeia (BP), diclofenac sodium is a white to slightly yellowish (off-white), slightly hygroscopic, crystalline powder[6]. It is sparingly soluble in water and alcohol; slightly soluble in acetone; freely soluble in methyl alcohol; practically insoluble in chloroform[7]. A number of analytical methods have been developed for the quantitative determination of this drug in dosage forms and in biological samples. However, HPLC-based methods proved to be more reliable as they can be used for the determination of percentage purity of raw material of diclofenac sodium and also in combination with other drugs[8]. A rapid, accurate, precise and reproducible UV spectrophotometric method for determination of diclofenac sodium in human stratum conium by skin stripping method using marketed diclofenac sodium topical formulations was explained[9]. In this method, diclofenac exhibited distinct λ_{max} in methanol at 285nm with linear relationship of r^2=0.9787between the concentrations of 5 and 25µg/ml.However this method may be challenging since it involves skin stripping. An accurate, rapid and precise flow extraction spectrophotometric method for determination of trace amounts of diclofenac sodium was proposed. The detection was performed at 282 nm againstphosphate buffer pH 6.4 as blank. The method was linear in the range of 3.0–80 µg/ml[10].Yilmaz *et al.*[11] have developed a HPLC method for the determination of diclofenac in human plasma.

Capillary electrophoresis is competitive to HPLC and other chromatographic methods, most especially when charged analytes have to be separated. Lachmann *et al.*[12]have reported a short and simple capillary zone

electrophoresis for determination of diclofenac in dosage forms using injection from the outlet sample tray and separation by reversed polarity. An alkaline borate buffer system containing 25nM sodium borate, pH 9.3 and UV detection of either 200/214 nm was used.

A simple HPLC method for the determination of diclofenac sodium in its pure form and in different pharmaceutical preparations was developed using mobile phase which was composed of a mixture of HPLC grade solvents (methanol, acetonitrile and deionized water) in the ratio of 60:20:20 respectively. Separation was completed within 2 min[13].A Thin layer chromatography (TLC) method was reported for the qualitative and quantitative analysis of diclofenac sodium tablets using a mobile phase prepared with environmentally-friendly solvents: toluene, acetone and glacial acetic acid. The R_f of diclofenac sodium was 0.60 and the method was repeatable and robust, with good selectivity and specificity[14].

Three sensitive, selective, and precise spectrophotometric methods based on manipulation of ratio spectra and validated for the determination of diclofenac sodium. The first method was based on ratio spectra peak to peak measurement using the amplitudes at 251 nm; the second method involves the first derivative of the ratio spectra ($\Delta\lambda$ = 4 nm) using the peak amplitudes at 326 nm and the third is the method of mean centering of ratio spectra using the values at 318 nm. The three methods were linear and the accuracy, precision, repeatability, and robustness are found to be within the acceptable limits[15].

Fotouhi*et al.*[16]developed electro membrane extraction (EME) and pulsed electro membrane extraction (PEME) coupled with HPLC for the extraction of diclofenac and investigated the effect of fundamental parameters on the extraction efficiency of both EME and PEME. Under optimized conditions, the results obtained showed that in comparison with EME, PEME has more effective micro extraction method, providing high extraction efficiencies in a short period of time and finally PEME was successfully used for the extraction of analytes from urine and plasma samples.

In the present study, a rapid and extraction-less method that utilized minimal total sample volume was developed for the quantitative determination of diclofenac sodium in pharmaceutical formulations based on complexation of diclofenac with manganese in mild acidic medium using one-factor-at-a-time (OFAT) optimization procedure.

2. 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).

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
Bateren Dexcel	0.21840	Diclofenac sodium	50
Dolo Meta B	0.64078	Diclofenac sodium	50
		Vitamin B_1	50
		Vitamin B_6	100
		Vitamin B_{12}	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

Reagents Used

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

Preparation of Chemicals and Reagents

All reagents and chemicals, including standard diclofenac, sample diclofenac solutions, potassium permanganate, sulphuric acid and perchloric acid solutions were prepared according to standard method of reagent preparations[17].

Diclofenac sodium (Standard solution)

Standard diclofenac sodium was freshly prepared by dissolving 4g in distilled water to give concentration of 4000mg/L (4 mg/mL). Working standard solutions of diclofenac sodium were freshly prepared from this stock 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.

Diclofenac sodium (Sample solution)

The average weights of diclofenac sodium (pharmaceutical preparation) 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.

Potassium permanganate

A stock solution of potassium permanganate 1580mg in water 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 prepared and used.

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 $2.5x10^{-9}$M, $5x10^{-9}$M, $7.5x10^{-9}$M, $1x10^{-8}$M, $1.25x10^{-8}$M, $1.5x10^{-8}$M, $1.75x10^{-8}$M and $2x10^{-8}$M were then prepared from this.

Perchloric Acid (0.1 M)

Exactly 8.5ml of perchloric acid was placed in a volumetric flask containing about 900ml of glacial acetic acid and mixed. Thereafter, 30ml of acetic anhydride was added and diluted to 1L with glacial acetic acid; it was mixed and allowed to stand for 24 hours.

Method Development

The method was developed by reacting diclofenac sodium and potassium permanganate in presence of sulphuric acid using OFAT optimization procedure; the reaction leads to formation of complex ion 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 – 700nm. 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

OFAT optimization is a procedure that is used to determine the influence of one factor on the response. This is done by varying the concentration of the optimized factor while other factors are kept constant. In the current studies the factors are diclofenac sodium, potassium permanganate, and sulphuric acid. First the initial concentration of the factors were selected (0.079mg/mL $KMnO_4$, 0.2mg/mL diclofenac and $2.5x10^{-9}$M acid), then diclofenac and sulphuric acid were kept constant while potassium permanganate was optimized until an optimum concentration was reached; next, the optimized concentration of potassium permanganate and the initial concentration of sulphuric acid were held constant while the concentration of diclofenac sodium was varied until an optimum concentration was reached. Then the optimum concentrations of both potassium permanganate and diclofenac were held constant while concentration of sulphuric acid was varied until an optimum concentration was reached. The three optimum concentrations were taken as the optimized conditions of the procedure. Sample vessels (plastics) of 10mL were used throughout the experiment. Deionized water was used to make up to mark (10mL) where necessary.

Method Validation

The method was validated using linearity, repeatability, reproducibility, limit of detection (LOD), limit of quantitation (LOQ), and accuracy (recovery) in accordance with International Conference on Harmonization (ICH) guideline[18].

Linearity and Calibration Curve

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

Precision

Intra-day precision was determined by performing experiments in triplicate at two different concentration levels within the same. for inter-day precision, however, analyte solutions were prepared in triplicate for five days to determine the reproducibility and the results were expressed as percent relative standard deviation (%RSD). From the calibration data standard, concentrations of 10mg/L and 30mg/L were chosen for carrying out both inter-day (reproducibility) and intra-day (repeatability) precision. The initial concentrations of diclofenac sodium, sulphuric acid and potassium permanganate were 500mg/L, $5X10^{-9}$Mand 1.580mg/mL respectively.

Limit of Detection (LOD) and Limit Quantification (LOQ)

In this work, LOD and LOQ were estimated using the following equations

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.

Accuracy

Tablets (10) from each brand of pharmaceutically prepared diclofenac sodium were ground into powder form, and an equivalent amount of 20mg/L of diclofenac sodium and the optimized concentrations of potassium permanganate 0.632mg/mL and $1x10^{-9}$M of sulphuric acidwere reacted together, a brown color complex was formed and allowed to stand 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 tablets used was dissolved in 30ml of glacial acetic acid and titrated with 0.1M perchloric acid; the end point was determined potentiometrically; 1ml of 0.1M Perchloric acid used is equivalent to 31.81mg of diclofenac sodium[6].

Statistical Analyses

Statistical analyses such as paired t-test and Chauvenet's criterion were used.A paired t-test is used to compare two population means where there are two samples in which observations in one sample can be paired with observations in the other sample[19]. In the current studies the paired t-test was used to compare OFAT optimization procedure with standard (B.P) method, while Chauvenet's criterion was used to ascertain the presence of outliers and possible counterfeit formulations.

3. RESULTS AND DISCUSSION

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 – 25 minute. Previous findings have put this value at the minimum of 15 minutes[20,21].

Reaction Mechanism

The reaction is based on redox reaction between diclofenac sodium and potassium permanganate in the presence of slightly acetic media which formed a brown color complex. The reaction was allowed to complete in 20 minute before taking the absorbance at a wavelength of 455nm. While lower concentrations of acid or alkaline pH would lead to a very slow reaction, higher concentrations of sulphuric acid would lead to the precipitation of the analyte which will affect absorbance and overall precision and accuracy of determination[22].

Using electron spin resonance scan, Sultan *et al.*[22]have found a stable sixtet hyperfine splitting which was previously attributed to manganese (II)[23]. These earlier researchers also found a weak triplet of sixtet hyperfine spliiting which was believed to correspond to the quinone imide form of the drug[24]; a super imposed radical feature peak was obtained and ascribed to the oxidized drug in a diradical species[22].

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 thedrug[22].

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).

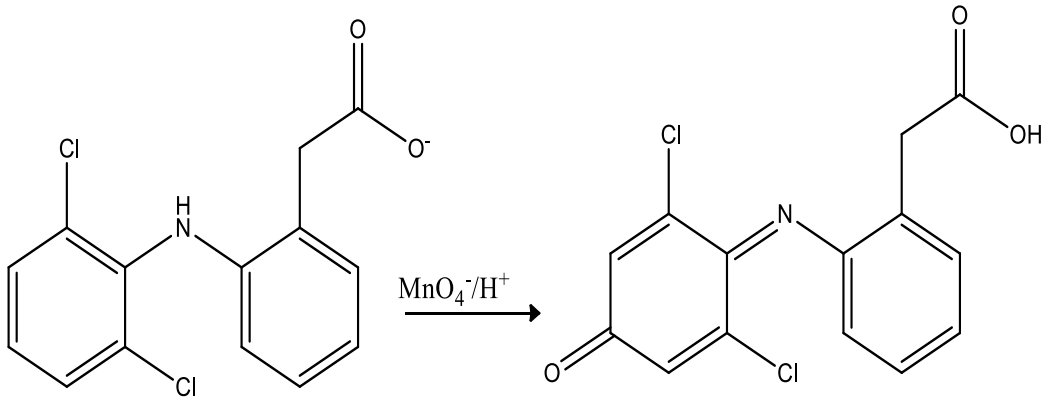

Figure 2: Proposed reaction scheme for the oxidation of diclofenac sodium with potassium permanganate in acidic media[22].

Absorbance Vs Wavelength Profile for Potassium Permanganate

The absorbance of potassium permanganate from 450nm to 700nm shows the maximum absorbance at 515 nm as shown in (Figure 3). This agrees with what was reported by earlier[25].

OFAT procedure

The level of potassium permanganate, diclofenac and sulphuric acid were optimized by OFAT optimization procedure (Figure 4 – 6) and the optimized conditions were 0.632mg/L potassium permanganate, 0.8mg/L of diclofenac sodium and 1X 10^{-9}M respectively (Table 2). This is in agreement with what was earlier reported[26,27].

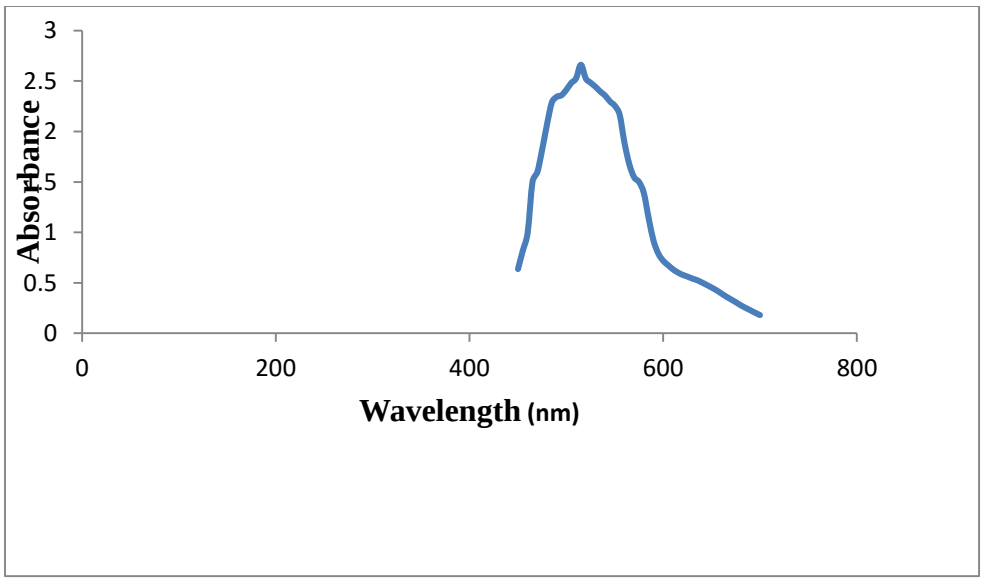

Figure 3: The UV scan of potassium permanganate.

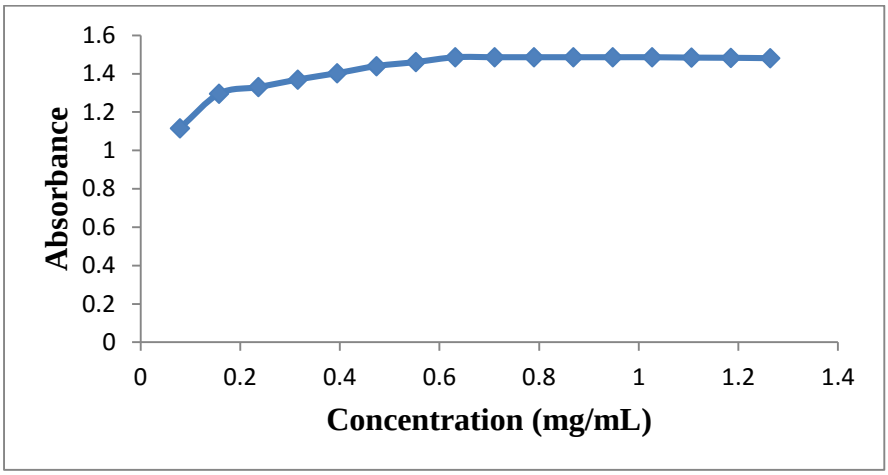

Figure 4: Absorbance vs concentration plot for potassium permanganate

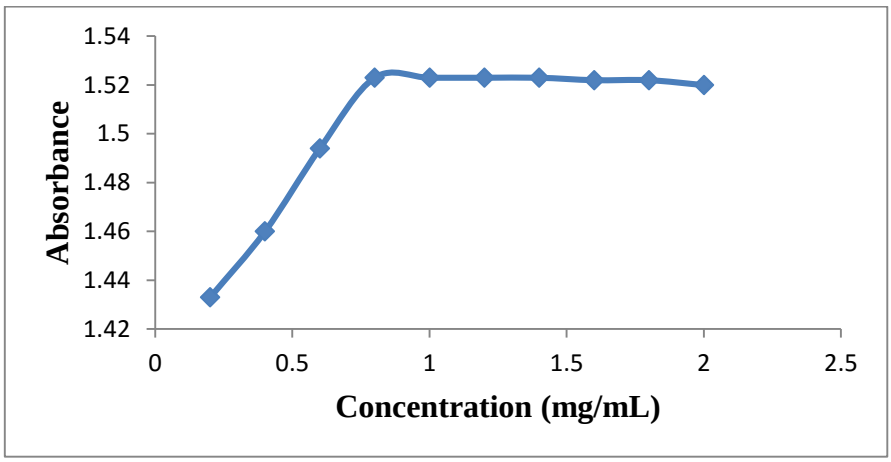

Figure 5: Absorbance vs Concentration plot for diclofenac sodium

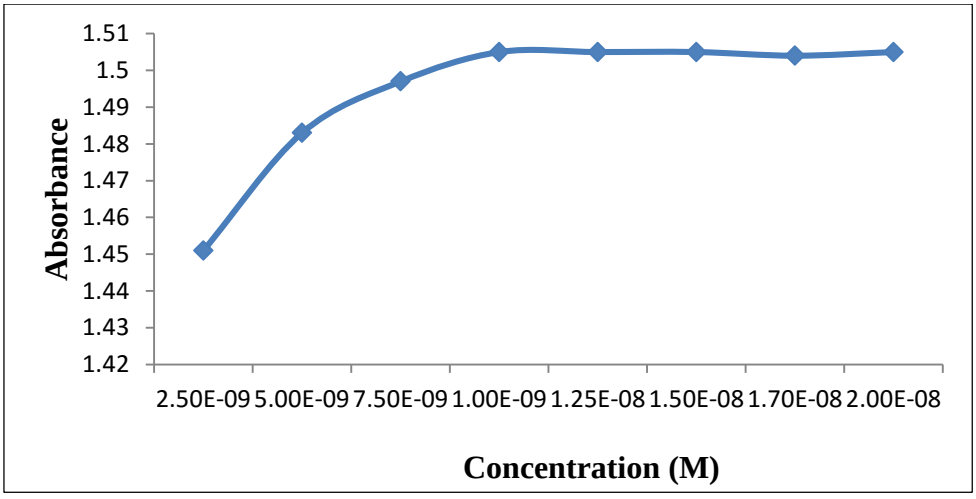

Figure 6: Absorbance Vs Concentration plot for sulphuric acid

Table 2: Optimized factor Levels

Factors	Factor level
Potassium permanganate	0.632mg/mL
Diclofenac sodium	0.8mg/mL
Sulphuric acid	$1x10^{-9}$M

Method Validation

The developed method was found to be linear with coefficient of determination, $r^2 = 0.994$ (Figure 7); this agrees with what was reported byThongchai*et al.*[28]. The method was found to be precise as both the intra – day (repeatability) and inter-day (reproducibility) precision have % RSD of 0.3% and 0.1% respectively(Table 3 and 4) and these agree with what was reported by Mahood and Hamezh[29]. The LOD and LOQ were found to be 0.13mg/L and 0.433mg/L respectively (Table 5) which agree with what was earlier reported[30]. Recovery (%) was found to be within the range of 85.86% - 110.53% (Table 6) and this is in agreement with what was reported by Ebeshi *et al.*[31].

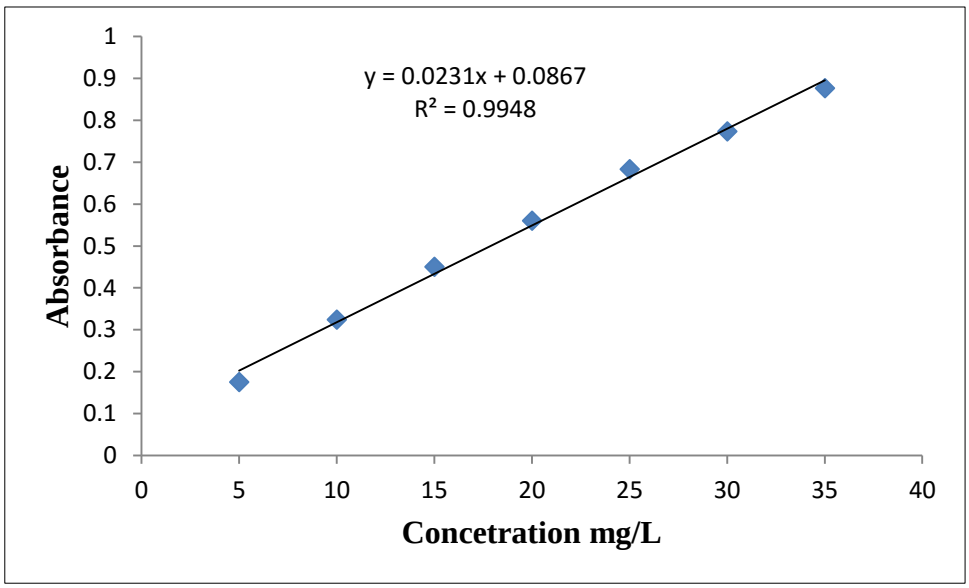

Figure 7: Calibration Curve

Table 3: Intra-day precision (repeatability)

Diclofenac, mg/L	Mean response	Standard deviation	%RSD
10	0.323	0.001	0.31
30	0.772	0.002	0.26
Average			0.29

Table 4: Inter-day precision (reproducibility)

Diclofenac, mg/L	Mean response	Standard deviation	% RSD
10	0.323	$3.2x10^{-4}$	0.09
30	0.773	$8.7x10^{-4}$	0.11
Average			0.10

Table 5: LOD and LOQ

LOD	0.130mg/L
LOQ	0.433mg/L

Table 6: Recovery (Accuracy) by OFAT optimization

Sample	Concentration used (mg/L)	Amount reported (mg)	Amount found (mg)	% Recovery
Dicloktis-plus	20	50	42.93	85.86
Sureclofen forte	20	100	103	103
Dicnac 550	20	50	40.5	81
Sorfen forte	20	50	8.435	16.87
Bateren Dexcel	20	50	52.5	105
Dolo Meta B	20	50	47.36	94.72
Voltaren	20	100	94.30	94.30
Clofenac	20	50	50.6	101.2
Olfen	20	50	44.94	89.88
Arthrotec	20	75	79.5	106
Lofnac 100	20	50	55.265	110.53

Recovery by Official Method

The recovery by official method (BP, 2013) was found to be within the range of 84.09 – 107.95 (Table 7). This agrees with what was reported by Kirim *et al.*[32]

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Table 7: Recovery (Accuracy) by Official Method

Sample	Concentration used (mg/L)	Amount reported (mg)	Amount yield (mg)	% Recovery[a]
Dicloktis-plus	20	50	43.37	86.74
Sureclofen forte	20	100	97.35	97.35
Dicnac 550	20	50	42.05	84.09
Sorfen forte	20	50	10.24	20.47
Bateren Dexcel	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	107.94

[a] according to British pharmacopeia, 2013

Statistical Analysis

From the statistical analysis, paired t – test shows that there was no significant difference between OFAT optimization and official method reported in British pharmacopeia (BP 2013) (Table 8). This is because t-Stat was less than t-Critical. However, Chauvenet's criterion (Table 9) applied to the procedures revealed that "sorten forte" was an outlier because $d_{max} > \tau_{max} X S_X$, and therefore, a possible counterfeit or sub-standard formulation[33].

Table 8: Comparison between OFAT optimization procedure and official method

	Variable 1	*Variable 2*
Mean	89.929	88.90827273
Variance	670.628379	580.1329438
Observations	11	11
Pearson Correlation	0.995343548	
Hypothesized Mean Difference	0	
Df	10	
t Stat	1.123046632	
P(T<=t) one-tail	0.143828134	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.287656268	
t Critical two-tail	2.228138842	

Variable 1: OFAT optimization procedure

Variable 2: Official method

Table 9: Chauvenet's criterion for OFAT optimization procedure

Sample	% Recovery	Mean	d_{max}	S_x	τ_{max}
Sorfen forte	16.87	89.85	72.98	25.85	2

Dicnac 550	81
Dicloktis-plus	85.86
Olfen	89.88
Voltaren	94.30
Dolo Meta B	94.72
Clofenac	101.2
Sureclofen forte	103.
Barteren Dexcel	105
Arthrotec	106
Lofnac 100	110.53

Advantages of Developed Method over the Standard Method

1. The time taken to carry out analysis using the new developed method was much faster as it took less than 30 minutes to complete the whole analysis compared to that of official BP method that required more than 24 hours to complete the analysis.
2. The proposed method used minimal total reaction cell volume of 10ml as against 30ml for the official method. Therefore, the proposed method is more environmentally-friendly and less wasteful.

4. CONCLUSION

A novel, simple, cost and time effective spectrophotometric analytical method for the determination of diclofenac sodium in pharmaceutical preparation was successfully developed. Statistical analysis showed that there was no significant difference between the accuracy of the developed method and official BP method. Nevertheless, the developed method has some advantages over the official method. Therefore, the developed method could effectively serve as an alternative to the contemporary method for routine determination of the analyte in pharmaceutical formulations.

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