International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com

Research Article

TITRIMETRIC AND UV-SPECTROPHOTOMETRIC DETERMINATION OF DICLOFENAC IN TABLET FORMULATION

Benjamin U Ebeshi*, Vaikosen N Edebi and Ossai Assuai
Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University, PMB 071, Wilberforce Island, Bayelsa State, Nigeria

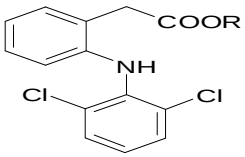
Received for publication: September 17, 2013; **Revised**: November 05, 2013; **Accepted**: December 13, 2013

Abstract: Diclofenac is a non-steroidal anti-inflammatory drug. It is usually formulated as a sodium or potassium salt. It exhibits anti-inflammatory, analgesic, and anti-pyretic activities both in animals and in humans. This study sets out to evaluate the quality and efficacy of diclofenac tablets using Chemical and UV spectrophotometric methods with a view to providing simple, sensitive and cost-effective analytical methods. The tablet samples were subjected to weight uniformity and hardness test. Method validation was by means of a precision assay. The methods were applied to the determination of diclofenac in tablets formulation. Six different brands of diclofenac tablets sourced from pharmacies in Yenagoa and Port-Harcourt, South-south region of Nigeria were analysed for diclofenac by non-aqueous titrimetry and UV spectrophotometry at the λ_{max} of 296 nm. All the six different brands of diclofenac tablet complied with the pharmacopoeia specification for uniformity of weight and the tablets possess suitable hardness for handling in manufacturing, packaging and shipping. The percentage purity of diclofenac from the non-aqueous titration ranged from 99.2 - 133%. The coefficient of variation for In-between run, Intra-day run and accuracy of the UV spectrophotometric method was within 4%. The percentage purity from UV determination ranged from 87.8 - 120.5%. Four of the six different samples conform to the BP specification using the non- aqueous titration while four of the brands did not meet the BP specification using the UV spectrophotometric method. The findings suggest that no single method could be adequate in the determination of the quality of pharmaceutical formulations.

Keywords: Titrimetry; UV-Spectrophotometry; Analytical methods: Diclofenac; Tablet Formulations

INTRODUCTION

Diclofenac is a non-steroidal anti-inflammatory drug (NSAIDs), a group of drugs of diverse chemical composition and different therapeutic potentials having a minimum of three common features: identical basic pharmacological properties, similar basic mechanism of action as well as adverse effects (Starek *et al.*, 2009). Diclofenac, which is chemically known as [2-(2, 6-dichloroamino) phenyl] acetic acid (Fig.1) is usually presented as a sodium or potassium salt. It exhibit anti-inflammatory, analgesic and anti-pyretic activities both in animals and human beings (Emdex, 2011). It is easily available and effective and thus extensively used by patients. The growing demand for this agents calls for higher level of quality control of its preparations so that they are in the highest possible degree free from any impurity that may come from the production process as well as from decomposition products of active or auxiliary substances (Todd and Sorkin, 1988).



Where, R = K: Diclofenac potassium; R = Na: Diclofenac sodium

Fig.1: Chemical structure of Diclofenac

***Corresponding Author:**
Dr. Benjamin U Ebeshi ,
Department of Pharmaceutical and Medicinal Chemistry,
Faculty of Pharmacy, Niger Delta University,
PMB 071, Wilberforce Island,
Bayelsa State, Nigeria.

The primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX). It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis (Dutta *et al.*, 2007). Inhibition of COX also decreases prostaglandin in the epithelium of the stomach, making it more sensitive to corrosion by gastric acid. This is also the main side effect of diclofenac. Diclofenac has a low to moderate preference to block the COX 2-isoenzyme and is said to have therefore, a somewhat lower incidence of gastrointestinal complaints than noted with indomethacin and aspirin.

Several methods have been proposed for the analysis of diclofenac. Sastry *et al.*, (1989) describe an accurate and precise spectrophotometric method for the determination of diclofenac sodium in bulk samples and pharmaceutical preparation with p-N, N-dimethyl phenylenediamine as solvent and maximum absorbance at 670nm. The reaction is sensitive enough to permit the determination of 2.0 – 2.4µg/ml. Bhatia *et al.*, (1995) proposed a procedure for simultaneous estimation of diclofenac sodium, chlorzoxazone and paracetamol in three component tablet formation. The

method is based on the native ultraviolent abosorbant maxima of the three drugs in 0.02M NaOH with diclofenac sodium producing absorbance maxima at 276nm. Perez *et al.,* (1997) also devised a new accurate, precise and reproducible method for the determination of diclofenac sodium in bulk and in pharmaceutical preparation using Eu (111) ion as the florescence probe. Agatonovic *et al.,* (1997) devised an accurate and precise spectrophotometric method in which diclofenac sodium is analyzed and determined as it's Fe (111) complex with chloroform as solvent and maximum absorbance at 481nm.

Garcia *et al.,* (2001) proposed a rapid, accurate and reproducible flourimmetric and spectrophotometric method for the determination of diclofenac in bulk samples and pharmaceuticals with sodium hydroxide solvent and measured at 455nm. Lala *et al.,* (2002) developed an accurate and reproducible method for the determination of diclofenac in human serum by HPTLC. Dencitometic analysis of diclofenac sodium was carried out at 280nm with diclofenac been detected at an Rf of 0.58. The extraction efficiency was found to range from 76–80%. Shaflee *et al.,* (2003) developed two modified method for assaying sodium diclofenac by GLC and HPLC. However, some of these methods are also highly expensive, hence this present study. The methods employed in this study are simple, sensitive and cost effective for the analysis of diclofenac in a resource limited setting.

MATERIALS AND METHODS

Materials:

Diclofenac reference standard (Sigma Aldrich, USA), glacial acetic acid (BDH), perchloric acid (BDH), sulphuric acid, distilled water, crystal violet, methanol (Sigma Aldrich), Acetic anhydride, Potassium hydrogen phthalate, and ethylacetate (BDH). Six brands of diclofenac sodium tablet were procured from Pharmacies in Yenagoa and Port-Harcourt, Niger Delta Region of Nigeria and were coded A to F. Their batch and official registration (NAFDAC) numbers and the address of the manufacturer for each brand as well as their corresponding manufacturing and expiry dates were duly documented.

Equipment:

These include analytical weighing balance (Galenkamp), burettes, UV spectrophotometer (Shimadzu, Japan), measuring cylinder, potentiometer (Galenkamp), volumetric flask, beakers, burettes, pipettes, conical flask, filter paper, weighing boat, pestle and mortar, mosanto hardness tester, and retort stands.

Weight uniformity test

Ten (10) tablet of each brand of diclofenac were accurately weighed one after the other using an analytical balance and the respective weights were recorded. The average weight, weight variation, standard deviation and percentage deviation of the respective brands were calculated.

Titrimetric assay:

Preparation of 0.1M Perchloric acid: Glacial acetic acid (0.9L) was measured into a volumetric flask after which 8.5ml of perchloric acid was added and the resulting solution was mixed thoroughly. 30ml of acetic anhydride was added to the content in the flask and then made up to 1000ml with glacial acetic acid. It was then mixed thoroughly and allowed to stand for 24 hours.

Preparation of potasium pthalate buffer: 2.042g of potassium hydrogen pthalate was dissolved in 50ml of water. 7.5 ml of 0.2M sodium hydroxide VS was added to it and then diluted to 200ml with water to give a solution of pH 4.4

Non-aqueous titration:

Standardization of 0.1M perchlorate: 0.2g of potassium hydrogen pthalate was weighed and dissolved in 30ml of glacial acid. A drop of crystal violet indicator was added and then titrated with 0.1M perchloric acid. The result was recorded and the procedure was duplicated.

Direct titration: An equivalent of 125mg of diclofenac was measured from each sample and dissolved in 15ml of glacial acetic acid and then filtered. A drop of crystal violet indicator was added to the filtrate and then titrated with 0.1M percholric acid. A blue to green colour change indicating the end point and the value was recorded. The experiment was repeated for each of the samples.

UV Spectrophotometric method:

Preparation of diclofenac stock solution: 100mg of standard diclofenac was accurately weighed and transferred into a beaker were it was dissolved with some basified methanol (methanol: 0.1M NaOH; 7:3 v/v). The resultant solution was transferred into a 100ml volumetric flask and it was made up to mark with the basified methanol solution to give a stock concentration of 1mg/ml. A portion of the solution was scanned at a wavelength between 200nm to 350nm.

Calibration curve for diclofenac:

Serial dilutions of the stock solution were made to give the following concentrations 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0µg/ml in the solvent. The UV absorbance of each concentration was taken at λ_{max} 296 nm. The graph of absorbance was plotted against concentration.

Method validation:

Precision and accuracy: The precision and accuracy of the UV spectrophotometric method were determined by performing five replicate analyses on the pure diclofenac solutions at three different concentrations (i.e. 1µg/ml, 5µg/ml and 10µg/ml). The In-between day precision was evaluated by running these concentrations five times within-run while the intra-day precision was performed by replicate analyses on the three drug concentrations for a period of five days with fresh solutions on each day.

Hardness Test: Ten (10) tablets from each brand were collected. Each tablet was placed between the spindle and the amil of the Mosanto hardness tester and pressure was by turning the knurled knob just sufficiently to hold the tablet in position. The reading of the pointer on the scale was adjusted to read zero and the pressure was increased as uniformly as possible until the tablet breaks. The hardness factor (average of several determinations) was calculated. This was done for samples A to F.

Statistical analysis:

Student t-test in the SPSS statistical software programme was used to compare the titrimetric and uv spectrophotometric assays in this study with $p < 0.05$ as the level of significance.

RESULTS

Weight uniformity test

The percentage deviation of each tablet from the average weight for the samples A–F ranged from approximately -4.7 to 3.6%.

Titrimetric Assay

Assay of diclofenac by non-aqueous titration: Titre values (Table 1) for sample A and the calculation for percent purity are shown below as an example. Samples B to F were similarly treated. The average titre values of 0.1M $HClO_4$ after titrations for samples A to F, the amount of diclofenac in the sample, percent purity and the standard deviation obtained from the visual titration are shown in Table 2. The percentage purity was calculated by taking the ratio of the calculated amount of the sample against the expected amount of the same sample and multiplied by a 100. The diclofenac tablets samples had % purity ranging from 99.2 to 133%.

Table 1: Titre values obtained from non-aqueous titration of Samples E to F

Sample	Titre values (ml)		Average titre value (ml)
	1ST	2nd	
A	4.5	4.3	4.4
B	4.0	3.7	3.85
C	6.1	6.1	6.1
D	4.7	4.8	4.85
E	4.5	4.3	4.4
F	4.7	4.4	4.55

Calculation for non-aqueous titration

Standardization of 0.1M $HClO_4$ using potassium hydrogen phthalate (KHP):

$$C_8H_5KO_4 + HClO_4 \rightarrow KClO_4 + C_6H_4(CO_2H)_2$$

204.22 $C_8H_5KO_4$ = 1000ml of 1M $HClO_4$
20.422 = 1000ml of 0.1M $HClO_4$
0.020422 = 1ml of 0.1M $HClO_4$

Factor

$$= \frac{Weight\ of\ Potassium\ Hydrogen\ pthalate}{titre\ volume\ X\ weight\ per\ ml}$$

$$= \frac{0.2}{11.43\ X\ 0.020422} = \frac{0.2}{0.2334} = 0.857$$

Determination of percentage purity of diclofenac samples:

Pure standard

Weight of sample = 125mg
Titre volume = 4.45
Factor = 0.857
1ml of 0.1M $KClO_4$ = 31.81mg of $C_{14}H_{10}Cl_2NNaO_2$

$$\% \text{ purity} = \frac{factor\ X\ titre\ value\ X\ weight\ per\ ml}{weight\ of\ drug}\ X\ 100\%$$

$$\% \text{ purity} = \frac{0.857\ X\ 4.45\ X\ 31.81}{125}\ X\ 100\% = 97.05\%$$

The calculation was repeated for sample A – F and the results are shown in Table 9

Table 2: Percent purity of Diclofenac Tablet samples by non-aqueos titration

Sample	Average wt of tablet taken (g)	Titre value (ml)		Average Titre (ml)	Amount of Diclofenac (mg)	% purity	% deviati
		1st	2nd				
A	0.2426	4.5	4.3	4.4	119.95	96	-4.21
B	0.2958	4.0	3.7	3.9	106.32	104.7	-17.57
C	0.402	6.1	6.1	6.1	166.29	133	24.83
D	0.3093	4.7	4.8	4.9	133.58	127.6	6.42
E	0.3006	4.5	4.3	4.4	119.95	96	-4.21
F	0.2175	4.7	4.4	4.6	125.40	99.2	0.32

UV Spectrophotometry:

Standard curve for Diclofenac:

The standard curve showing the absorbance versus concentration of diclofenac at the wavelength of 296nm is shown in Fig.2. The standard curve was linear over a concentration range of 0.5 to 16 µg/ml with the regression line equation obtained as y = 0.071x + 0.060, which was in line with the Beer-Lambert's law.

Precision of the analytical Method:

The coefficient of variation, which is a measure of the precision, was < 3% for both In-between run and the Intra-day run, which is a measure of reproducibility of the method for diclofenac (Table 3). Also, the relative error (%), an indicator of accuracy was less than 4%.

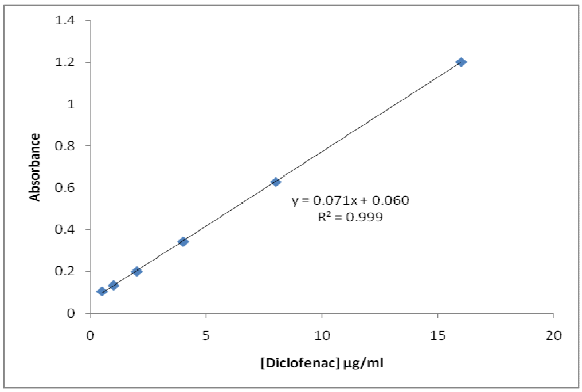


Fig.2: Calibration curve for Diclofenac

Table 3: Precision and accuracy studies for diclofenac (n=5)

	Expected conc. (mg/ml)	Observed mean conc. ± SD (µg/ml)	Coefficient of variation (%)
In-between run	1.0	0.96 ± 0.024	2.5
	5.0	5.15 ± 0.064	1.2
	10.0	9.95 ± 0.066	0.6
Intra-day run	1.0	0.98 ± 0.026	2.65
	5.0	5.05 ± 0.057	1.13
	10.0	9.68 ± 0.091	0.94
Accuracy	1.0	1.04 ± 0.034	3.26
	5.0	4.97 ± 0.048	0.97
	10.0	10.13 ± 0.082	0.81

Percentage purity for diclofenac test samples

The percent purity of samples A to F (Table 4) was calculated using the regression equation obtained from the standard with sample calculation as shown below:
Using the regression equation, y = 0.071x + 0.060 from the calibration curve the actual concentrations of the various brands were calculated as shown below.

Where; x = actual concentration
y = absorbance
For brand A; At 4 µg/ml, absorbance = 0.322

$$x = \frac{y - 0.06}{0.071}$$

$$x = \frac{0.322 - 0.06}{0.071}$$

$$x = \frac{0.262}{0.071}$$

$$x = 3.69\ \mu g/ml$$

$$\text{Percentage purity} = \frac{\text{Observed concentration}}{\text{Expected concentration}} \times 100$$

$$\% \text{ purity} = \frac{3.69 \times 100}{4}$$

$$\% \text{ purity} = 91.25\%^{w}/_{v}$$

The same calculation was carried out for the other brands and the results are shown in the table above.

Table 4: Percentage purity of Samples A to F

Sample code	Absorbance 4 µg/ml	Observed conc. (µg/ml)	Percentage purity (%)	% deviation
A	0.322	3.69	91.25	-8.40
B	0.354	4.14	103.50	3.38
C	0.402	4.82	120.50	17.01
D	0.381	4.52	113.00	11.50
E	0.309	3.51	87.80	-13.96
F	0.329	3.80	95.00	-5.26

Hardness test:

Table 5: Hardness test for diclofenac sample A - F

S/NO.	Hardness, H (kg)					
	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
1	10.5	9.0	9.0	7.5	15	4.0
2.	10.5	9.5	7.0	6.5	14.5	3.5
3.	10.0	9.5	9.0	7.0	15.5	4.5
4.	11.0	12.5	8.5	6.5	14.5	10.0
5.	10.0	12.5	7.0	7.5	14.5	11.0
6.	10.5	10.5	7.0	7.0	14.5	6.0
7.	11.0	10.5	10.0	7.0	14.5	6.0
8.	10.5	7.5	9.5	7.0	14.5	5.0
9.	10.5	10.5	10.0	7.0	12.5	6.5
10.	12.0	7.5	10.0	7.0	15.5	5.5
Mean (kg)	10.65	9.95	8.7	7.0	14.55	7.0

DISCUSSION

Standards for uniformity of weight are applied to tablets and capsules, which are supplied in unit dose form because they are subject to more variations than comparable preparations supplied in multi dose forms. For tablets with average weight above 80mg and less than 250mg, the percentage deviation from the average weight permissible in the BP, 2008, is 5%.

The six different brands (brands A-F) of diclofenac tablets passed the test for uniformity of weight. This is because none of the tablets individual percentage deviation exceeded 5%. The implication of a tablet deviating by more than 5% from the average weight is an increase in the quantity of the active ingredient above average and this could result in increased plasma

concentration of such tablet above the maximum safe concentration when administered. It is therefore important to carry out the uniformity of weight test in order to assess the uniformity of the content of the active ingredient in each unit dose. Suitable hardness of tablet is necessary for handling in manufacturing, packaging and shipping (Gaud *et al.,* 2000). The hardness factor (average of several hardness determinations) obtained showed that all the tablets have suitable hardness.

From the non-aqueous titration carried out, it was observed that four of the different brand of diclofenac fell within the official limit of 95 – 105% (BP, 2008). Sample C and Sample D with percentage purity of 133% and 127.6%, respectively, did not conform to the official limit stated by the BP.

Standard diclofenac was scanned within the UV–VIS region for maximum wavelength (lambda) of absorption which was found to be 296nm. The calibration curve for reference diclofenac was linear over a concentration range of 0.5 to 16.0 µg/ml with the regression line equation obtained as y = 0.071x + 0.060, which is in conformity with the Beer-Lambert's law. The regression coefficient of (R^2=0.999) allowed for accurate reading of the concentrations of all the test samples. The coefficient of variation (%), an indicator of precision and the relative error (%), a measure of accuracy of the analytical method, which were evaluated by replicate analyses of the pure drug solution at three different concentrations within working range, indicates high precision and accuracy of the method. The intra-day precision, which is a measure of the reproducibility of the method with coefficient of variation being less than 3% shows that the method was highly reproducible. The spectrophotometric method was therefore sensitive and reproducible.

The assay of samples A-F by UV spectrophotometric method gave results that showed that not all the sample fell within the BP range. This may imply that not all the sample contain up to the required active ingredient as specified by the BP. Samples B and F fell within the BP range while sample A, C, D and E fell outside the BP range. The samples which fell above or below the required standard as stated by the BP could be said to be sub-standard. Those that fell above the upper limit could be said to be sub-standard on account of overage. The implication of overage of this nature is grave since drug products are potential poison and therefore when administered at dosages exceeding their limits may predispose patients to adverse drug reactions. The findings of sub-optimal amount and overage in the test samples may stem from under incorporation or over-incorporation of active principles to probably beat the accelerated stability testing, poor formulation, poor storage facilities, adulteration and possible inefficiency of the UV spectrophotometer.

On comparing the spectrophotometric and titrimetric methods, it was observed that samples C and D failed to meet the requirement for both methods while samples A and E passed the titrimetry method but failed to meet the stated standard using spectrophotometric analysis.

The findings in this study showed that various in-house and modified chemical and instrumental methods of analysis including non-aqueous titration and UV spectrophotometry (Landsdrop *et al.,* 1990; Reynolds, 1993; Hinz *et al.,* 2005) are effective in the determination of the quality and quantity of active substances in diclofenac tablets. Based on the results obtained from these methods, it is very important to combine various simple, precise, and sensitive methods of analyses to authenticate the quality of drug sample because of error and limitation of some methods. When a drug conforms to standards as stated in the official monograph, it gives assurance of the quality and predicts therapeutic efficacy as well as safety of the drug. It is therefore necessary for both manufacturers and regulatory bodies to utilize more than one analytical method in the determination of the quality of active drug in pharmaceutical preparations.

CONCLUSION

In conclusion, diclofenac can be successfully analyzed using titrimetric and UV spectrophotometric methods. The use of various simple, precise, and sensitive methods in combination for the determination of active drug in pharmaceutical formulation is very essential to authenticate processes especially in resource limited environment. Therefore, no single method applied in isolation is sufficiently accurate in providing enough data or information on the quality of a drug product.

REFERENCES

1. Agatonovic KC, Zivanovic L, Zecevic M, Radulovic D (1997). Spectrophotometric study of diclofenac-Fe (III) complex. J. Pharm. Biomed. Anal., 16, 147-153.

2. Carter S.J (1996). Cooper and Gunn's Tutorial Pharmacy, 6th edition, CBC Publishers and Distributors 4596/1A, 11 Darya Ganj, New Delhi India. P 15

3. Dutta NK, Annadurai S, Mazumder K, Dastidar SG, Kristiansen JE, Molnar J, Martins M, Anaral L (2000) "The anti bacterial action of diclofenac shown by inhibition of DNA synthesis. Int. J. Antimicrob. Agents 14(3): 249-251

4. Dutta NK, Annadurai S, Mazumder K, Dastidar SG, Kristiansen JE, Molnar J, Martins M, Amaral L (2007). Potential management of resistant microbial infections with a novel non-antibiotic: the anti-inflammatory drug

diclofenac sodium. Int. J. Antimicrob. Agents 30(3): 242-249

5. Emdex (2011). The complete drug formulary for Nigeria's health professionals. Non- steroidal anti-inflammatory drugs. Lindoz Books Int'l 6040 Montovideo Road, Unit 48, Mississauga, Canada. pp 66-69

6. Fowler PD, Shadforth MF, Crook PR, John VA (1983). Plasma and synovial fluid concentrations of diclofenac sodium and its major hydroxylated metabolites during long term treatment of rheumatoid arthritis. Eur. J. Clin. Pharmacol. 25 (3): 389-94

7. Garcia MS, Albero MI, Sanchez PC, Molina J (1998). Simpler and faster determination of diclofenac tablets. J. Pharm. Biomed. Anal. 17, 267- 273.

8. Garcia S, Sanchez PC, Albero I, Garcia C (2001). Spectrophotometric, chromatographic and spectrofluorometric methods for determination of diclofenac. Microchim. Acta. 3, 67-71.

9. Hinz B, Chevts J, Renner B (2005). Bioavailability of diclofenac potassium at low doses. Br J Clin Pharmacol. 59(1), 80-84.

10. Lala LG, Mello P, Naik SR (2002). HPTLC determination of diclofenac sodium from serum. J. Pharm. Biomed. Anal. 29, 539-544

11. Landsdorp D, Vree TB, Janseen TJ, Guelen PJM (1990). Pharmacokinetics of rectal diclofenac and its hydroxyl metabolites in man. Int J Clin Pharmacol Ther Toxicol. 28 (7): 298-302

12. Mascher H (1989). The pharmacokinetics of a new sustained-release form of diclofenac sodium in humans. Drug Design and Delivery. 4 (4): 303-311.

13. Perez RT, Martınez LC, Sanz A, Miguel MTS (1997). High pressure liquid chromatographic analysis of urea herbicides in foods. J. Pharm. Biomed. Anal.16, 249-254.

14. Riess W, Stierlin H, Degen P, Faizle JW, Gerardin A, Moppert Sallmann A, Schmid K, Schweizer A, Sule M, Theobald W, Wagner J (1978). Pharmacokinetics and metabolism of the anti-inflammatory agent Voltaren. Scandinavian Journal of Rheumatology. 22(S), 17-29.

15. Reynolds JEF (1993). Martindale, The extra pharmacopoeia, 30th ed., The Pharmaceutical Press, London, p. 11.

16. Sastry CP, Prasad T, Suryamarayana MV (1989). Extractive spectrophotometric determination of some anti-inflammatory agents with methylene violet. Analyst, 114, 513-5

17. Sastry CP, Suryanarayana MV (1989). Spectrophotometric determination of diclofenac sodium. Microchem. J., 39, 277-282

18. Shaflee A, Amini M, Hajmahmodi M (2003). Chromatographic and spectrofluorometric determination of diclofenac. J. Sci. Islam. Repub. Ira, 14, 21-25.

19. Starek M, Krzek J (2009). A review of analytical techniques for determination of oxicams, nimesulide and nabumetone. Talanta, 77 (3): 925-42.

20. The British Pharmacopoeia (2008). Her Majesty Stationery office England, Pharmacopoeia commission, 3: A126-A127.

21. Todd PA, Sorkin EM (1988). Diclofenac sodium, A reappraisal of its pharmacodynamic and pharmacokinetics properties, and therapeutic efficacy. Drugs,35(3), 244-285.

Source of support: Nil
Conflict of interest: None Declared