# New method for determination of diclofenac sodium by High Performance Liquid Chromatography

**Riyadh Ahmed Atto**

**Department of Pharmaceutical Sciences, College of Pharmacy, University of Mosul, Mosul, Iraq**

***Received 26/12/2011 Accepted 9/2/2012***

## Abstract

In this study a simple, coast effective and direct simple High Performance Liquid Chromatography (HPLC) method has been developed for the determination of diclofenac sodium in it's pure form and different pharmaceutical preparations. Standard diclofenac sodium and its dosage forms were supplied from Ninava State Company For Drug Industries and Medical Appliances (NDI). HPLC method was developed by using mobile phase which was composed of a mixture of HPLC grade (methanol, acetonitrile and deionized water)in the ratio of (60:20:20) respectively. Separation has been completed within 2 min. Calibration curve was linear, coefficient correlation was found to be 1.00 at a concentration of (0.25-4.0 $\mu g.ml^{-1}$). The relative standard deviation (RSD) was found to be <1.2%. The proposed method was successfully applied for the determination of diclofenac sodium in its pure form and different pharmaceutical preparations (injection, tablets, eye drops, suppositories and gel) without using buffer system and there is no inference with additives.

**رياض احمد عطو**

الملخص

في هذه الدراسة، تم تطوير طريقة جديدة وسهلة و فعالة واقتصادية ومباشرة لتقدير كمية الدايكلوفيناك صوديوم في شكله الخام وفى أشكاله الصيدلانية المختلفة بواسطة جهاز الأستشراب عالي الأداء . تم تجهيز مادة الدايكلوفيناك صوديوم القياسية ومستحضر اته الصيدلانية مِنْ الشركةِ العامة لصناعة الأدوية والمستلزمات لطبية في نينوى(إن دي آي ). تمت عملية الأستشراب باستخدا م الطور المتحرك والمتكون من مزيج عالي النقاوة من (ميثانول،أسيتونايترايل،والماء اللاأيوني) وبنسبة (60:20:20) على التوالي . تمت عملية الفصل بنجاح خلال 2 دقيقة. منحنى التحديدِ كانَ خطيَّ، عاملِ الارتباط وُجِدَ لِكي يَكُونَ 1.00 في تركيز (0.25-4.0 مايكرو غم .مل$^{-1}$ ). الانحراف المعياري النسبي (آر إس دي) وُجِدَ لِكي يَكُونَ> 1.2 %. الطريقة المُقتَرَحة تم تطبيقها بنجاح لتقدير كمية الدايكلوفيناك صوديوم في شكلِه الخام وأشكاله الصيدليةِ المختلفةِ (كالحقن و الحبوب و قطرات العين والتحاميل والهلام) بدون استعمال نظامِ حاجزِ وليس هناك استدلال بالإضافاتِ.

## Introduction

Diclofenac sodium, or Sodium [O-(2,6-dichlorophenyl)-amino-phenyl]acetate (Fig. 1) is a non-steroidal antiinflammatory analgesic with potent cycloxygenase inhibition activity [1-5]. This drug is commonly used for pain control and treatment of rheumatic diseases [4,5].

***Figure(1):- Diclofenac sodium*[2]**

Several procedures have been described for the determination of diclofenac sodium in pharmaceutical preparations. These procedures include reports UV-Visible spectroscopy[6,7] chemometry[8] spectrofluorometry[9], High performance liquid chromatography[10-13], titrimetry[14] . potentiometry[15-18] and polarographic analysis [19]. Some of these procedures are cumbersome and too costly for routine analysis, some others use buffer system and others consume a comparatively long time for analysis about (17 min.) by using C18 column as in B.P. The aim of this study is to develop a simple and rapid liquid chromatographic method for determination of diclofenac sodium in its different dosage forms (injection, eye drops, tablets, suppositories and gel) with a retention time of only 2 minutes using the RP (C8 ) column without using buffer system.

## Materials and Instruments

### Materials

Reference standard of diclofenac sodium (99.98) % was of analytical or pharmaceutical grade which was supplied from Nineveh Drug Industry (Iraq). It was used without further purification. All other solvents (acetonitrile, methanol and deionized water) were of HPLC grade supplied from Fluka-company (Germany) and used throughout.

### Instruments

1. The ultraviolet spectra were obtained via Carrywinn U.V. Varian U.V. – visible spectrophotometer (Australia).
2. HPLC Shimadzu. Intelligent LC pump with sampler programmed at 20 µl capacity per injection was used. A three port valve Model was used as a venting valve. The detectors used were a LC-2010 monitor operating at 283nm.The C8 (15cmx4.6mm) of 5 µm internal diameter analytical columns were constructed from supelco USA.

## Analytical method

A series of working standard solution containing (0.25-4.0 $\mu g.ml^{-1}$) of diclofenac sodium and the sample solution of pharmaceutical preparation were prepared . A 20 µl a liquot of the solution was injected on to the column in a duplicate and the chromatograms were recorded.

**Procedures for pharmaceutical preparations:**

For the determination of diclofenac sodium, the standard solution was

prepared by dissolving (25 mg) of diclofenac sodium in (25 ml) methanol which was then transferred into the volumetric flask and the volume was filled up to (10 ml.) with the mobile phase, then (2.5 ml.) was taken from the last one and was further diluted with the mobile phase up to (100 ml.) to produce the working standard concentration of (0.025 $mg.ml^{-1}$). These solutions were preserved at (25 °C) in alight resistant containers and were left to attain room temperature before use.

**Test preparation:**

**Ampoules**

A quantity equivalent to 25 mg (1.0 ml.) of diclofenac sodium injection (25 $mg.ml^{-1}$) was transferred into the volumetric flask and the volume was filled up to (100 ml) with methanol, then (1.0 ml) was taken and further diluted with mobile phase to (10 ml), filtered and applied to HPLC system for the analysis.

**Tablets**

Ten tablets each containing (25 mg.) of diclofenac sodium were crushed in a mortar and pestle into fine powder. An amount of the powder equivalent to (50mg) of pure diclofenac sodium was accurately weighed, after that dissolved in a minimum volume of methanol. The solution was stirred for (15 min.) and then made up (50 ml.) with methanol. The combined extracts were transferred to a volumetric flask then we take (1 ml.) and dilute to (10 ml.) with mobile phase. From this solution, (2.5 ml.) was piped and transferred to (10 ml.) volumetric flask and made volume up to the mark with mobile phase to get the concentration (0.025µg.$ml^{-1}$) of diclofenac sodium.

## Eye drops

A quantity equivalent to 1ml. (1mg. $ml^{-1}$.) of the eye drop solution was taken and transferred into the volumetric flask. The volume was filled up to(10 ml.) with methanol, then (2.5 ml.) of this solution was transferred into a volumetric flask the volume was further diluted up to the mark in a volumetric flask to (10 ml.)with mobile phase, which will then be applied to the C8 column.

## Suppositories:

The weight of five suppositories (100 mg./suppository)was transferred to a porcelain dish, melt and allow cooling while stirring with a glass rod. Accurately weigh a (50 mg.)of the melted diclofenac sodium, extract with (50 ml.) methanol, from this filtered solution (1 ml. ) was piped and diluted up to (10 ml.) then (2.5 ml.) of this solution was further diluted with mobile phase up to (10 ml.).

## Gel:

We dissolved 5 g. of (1 % w/w) diclofenac sodium gel in (30 ml.) of methanol, this sample was subjected for vigorous shaking for about (30 min) for complete extraction of drug and then it was kept in an ultrasonic bath for (15 min.) and then was filled up to (50 ml.).After this process, the sample was centrifuged for (10 min.) at (3600 $min.^{-1}$),then we take (1 ml.) and dilute it up to to (10 ml.) with mobile phase. After that (2.5 ml) from this sample were further diluted up to (10 ml.)to get the concentration (0.025µg.$ml^{-1}$) of diclofenac sodium.

## Results and Discussion

The development of HPLC methods for the determination of drugs has received considerable attention in recent years because of their importance in the quality control of drugs and pharmaceutical products. Column chemistry, solvent type, solvent strength (volume fraction of organic solvents in the mobile phase, detection wavelength, temperature and flow rate were varied to determine the chromatographic conditions giving the best separation as shown in(table (1)).

**Table( 1):- HPLC conditions**

| **Column** | **Supelco C8 (150cm,4.6mm),5µm** |
|---|---|
| Detector | UV visible detector. |
| Mobile phase | Methanol, Acetonitrile and deionized Water |
| Wave length | 283 nm. |
| Retention time | 2.0 min. |
| Flow rate | 1.0 ml/min. |
| Temperature | Ambient |
| Injection volume | 20 µl. |

Different mobile phases containing various ratios of methanol, acetonitrile and deionized water were examined using different columns. The elute consisted of (methanol, acetonitrile and deionized water) in the ratio of (60:20:20, V/V/V) respectively was selected and maintained at a flow rate of (1 ml. min $^{-1}$) applied in C8 column (15cm x 4.6mm) packed with 5 µm supelco, were found to be as optimal for obtaining well defined and resolved peaks. After passage of (20 µl) of eluent, the valve was switched for elution of diclofenac sodium into the analytical column C8. The elute was detected at a wave length of (283 nm.) and it was found to be the optimum wavelength for detection and quantification of diclofenac sodium. Calibration curve was constructed by plotting the mean peak area versus the concentration of diclofenac sodium. The area of the chromatographic peak as shown in figure (2) was measured [20] and the concentration of diclofenac sodium obtained by comparing with working standards. With these optimized chromatographic conditions, typical chromatogram of diclofenac sodium (Fig. 2) was obtained and was found to be a very sharp peak with better resolution within the retention time ($t_R$) 2.0 min. In contrast other papers used buffer system or consume much longer time than this method or use diclofenac derivatives [21].

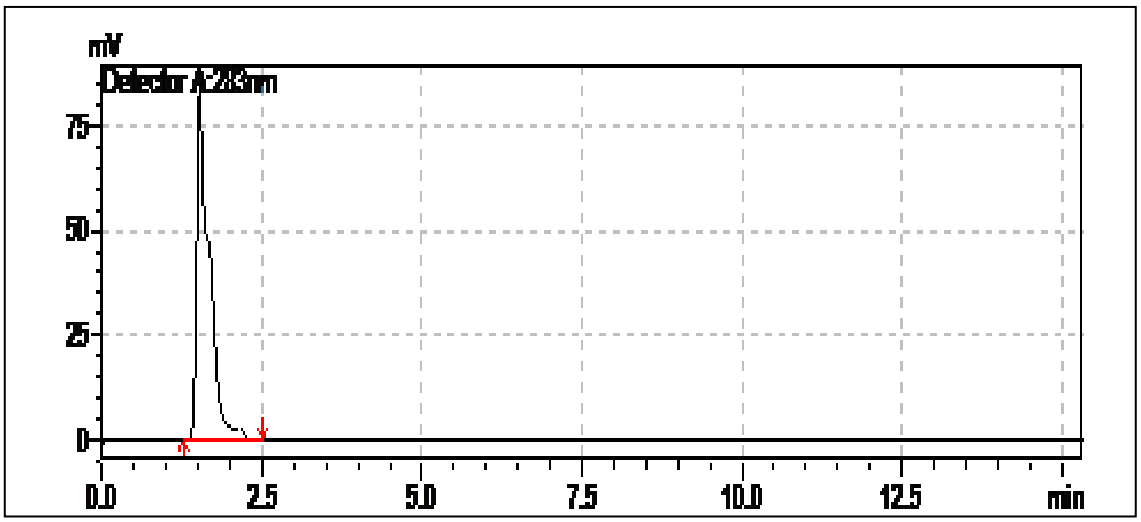


**Figure (2): Typical Chromatogram (diclofenac sodium (10 µg.ml$^{-1}$))**

The retention of diclofenac sodium increase when the ratio of the eluent mixture was set at 60:20:20 (v/v/v) of methanol, acetonitrile and deionized water respectively and decrease when the ratio otherwise change to any other ratio .This is due to a mpetition of binding to a solid phase between diclofenac sodium and the eluent mixture ion time. the calibration curve was found to be linear over the concentration range(0.25-0.4 µg.ml$^{-1}$). This linearity was determined for diclofenac sodium by plotting peak area against concentration. From these calibration plots it was clear that the response was a linear function of concentration over the range of (0.25-0.4 µg.ml$^{-1}$) for diclofenac sodium. The linear regression equations for diclofenac sodium were found to be:

Diclofenac sodium y=0.6x + 46.87(n=5, $r^2$ = 1.0), where y is the response (peak area) and x is the concentration in µg.ml$^{-1}$.

So, that the calibration curve of diclofenac sodium was found to be linear with a correlation of 1.00 as shown in (Fig.3).

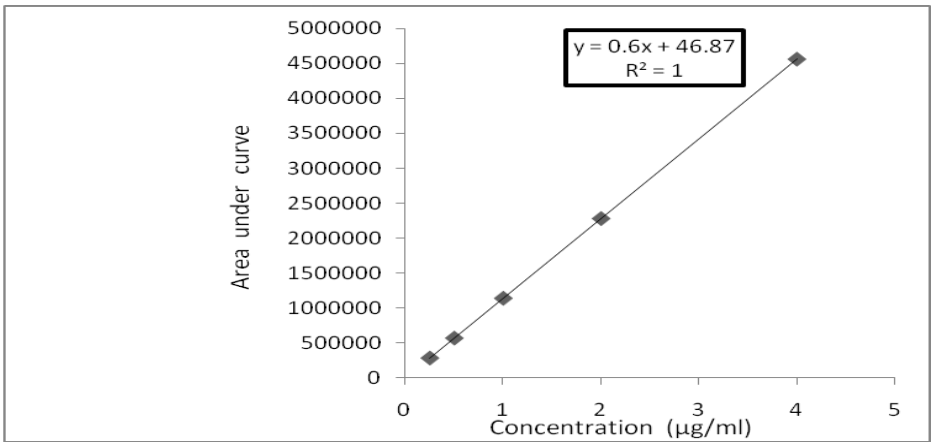


***Figure (3): Calibration curve of diclofenac sodium.***

The concentration of the unknown had been constructed from the calibration graph or calculated from the regression equation derived from the concentration and peak area data. This liquid chromatographic method is capable of analyzing a large number of samples in a single day. The same mobile phase was used throughout the experimental work and no interference peak from any excipient was observed indicating that the excipient didn't interfere with the estimation of diclofenac sodium by the proposed HPLC method. These results show that the method could find practical application as a quality control tool for analysis of diclofenac sodium from their different pharmaceutical dosage forms in quality control laboratories.

**Precision and Accuracy:**

The precision and accuracy of the assay were determined by repeatability (intra-day)and intermediate precision (inter-day). This method was used for its intra inter day precision, the relative standard deviation based on the peak area for five triplicate injections were

found to be between (0.6 and 1.18). n= 5) was expressed as a relative standard deviation and range between (0.19% and 1.15%) (Table 2) .

The inter assay precision (3 days

**Table (2):- inter and intra day precision of diclofenac sodium assay by the proposed HPLC method .**

| Concentration of diclofenac sodium in µg.ml$^{-1}$ | Observed concentration of diclofenac sodium | |
|---|---|---|
| | Intra day* RSD % | Inter day* RSD % |
| 1 | 1.18 | 1.15 |
| 2 | 0.97 | 1.01 |
| 4 | 0.6 | 0.91 |

***Average of five determinations***

Repeatability was evaluated by assaying samples, at same concentration and during the same day. The intermediate precision was studied by comparing the assays on different days. Five sample solutions were prepared and assayed.

.

The obtained results are presented in (table 3) which reveals that there is a close agreement between the results obtained by the proposed HPLC method and the label claim for the determination of diclofenac sodium in pharmaceutical preparations

**Table (3):- Results of analysis of formulations and recovery of diclofenac sodium.**

| Pharmaceutical formulations | Labeled amount | Found amount | % Recovery |
|---|---|---|---|
| Diclofenac Ampoule | 25mg/ml | 24.248 | 96.99 |
| Diclofenac Tablet | 25mg/ml | 24.67 | 98.86 |
| Diclofenac Eye drop | 1mg/ml | 0.978 | 97.80 |
| Diclofenac Suppository | 100mg/supp. | 100.2 | 100.02 |
| Diclofenac Gel | 1% w/w in a gel | 0. 98 % | 98.00 |

## Conclusion

A new HPLC method has been developed for analysis of diclofenac in its different pharmaceutical formulations. As shown above that the proposed method was of low cost, effective, accurate, reproducible, repeatable, linear, precise, and selective, proving the reliability of the method. The proposed method also indicates a comparative less time consuming method developed by selecting a solvent system without buffer i.e. this method retorts with elution of the sample from C8 column within (2.0 min.), which enables rapid quantitation of many samples in routine and quality control analysis of

injection, tablets, eye drops, suppositories and gel formulations. In addition to that the same solvent was used throughout the experimental work and no interference from any excipient was observed.

**Applications:**

This method was successfully applied to analysis of diclofenac sodium in its different formulations making its use as a reliable and advantageous alternative to other methods for routine methods for analysis of diclofenac sodium.

**Acknowledgment**

I'd like to thank keen interest the chemist Suhaib N. Lottfi (responsible for quality control in Ninava State Company For Drug Industries and Medical Appliances (NDI), Mosul-Iraq.) and Dr. Naief for constant advising me and for his valuable suggestions and encouragement from time to time and also wishes to thank for industries, who were provided free sample drug for the work.

## References

1.The United State Pharmacopeia USP 28, United States Pharmacopeia l Convention, INC. Asian Edition; 2009:2124.

2. Furst DE, Munster T. Non-steroidal anti-inflammatory drugs, disease modifying ant rheumatic drugs, non-opioid analgesics and drugs used in gout. In: Katzung BG. Basic and clinical pharmacology, 8th ed. New York, Mc Graw – Hill, 2006; 598-99, 604.

3.Ku EC, Wasvary JM, Cash WD. Diclofenac sodium (GP 45840, Voltaren), a potent inhibitor of prostaglandin synthetase. *J.Biochem. Pharmacol*. 1985; 24: 641–643.

4. Menasse R, Hedwall PR, Kraetz J, Pericin C, Riesterer L, Sallmann A, Ziel R, Jaques R. Pharmacological properties of diclofenac sodium and its metabolites. *Scand. J. Rheumatol*. 1978; 22:5–16.

5.Brogen RN, Heel RC, Pakes GE, Speight TM, Avery GS. Diclofenac sodium: a review of its pharmacological properties and therapeutic use in rheumatic diseases and pain of varying origin. *Drugs*.1980;20(1): 24–48.

6.British Pharmacopoeia Commission. International edn. HMSO publication, London;2007;1:469.

7.Sultan S., Mohamed H., Jabar A. and Alarfaj N. A new analytical form for the spectrophotometric determination of low levels of diclofenac. *J. Flow Injection Anal*.2010;27:49.

8. Castellano, P.M., Vignaduzzo, S.E., Maggio, R.M. and Kaufman, T.S. Application of a chemometric method for simultaneous determination of acetaminophen and diclofenac in content-uniformity and drug-dissolution studies. *Anal. Bioanal. Chem*. 2005;382: 1711-1714.

9.Arancibia, J. A., Boldrini, M. A. and Escandar, G. M. Spectrofluorimetric determination of diclofenac in the presence of α-cyclodextrin. *Talanta*. 2000;52(2): 261-268.

10.Nebot, C., Gibb, S. W. and Boyd, K. G. Quantification of human pharmaceuticals in water samples by high performance liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta*. 2007;598: 87-94.

11.Panusa, A., Multari, G., Incarnato, G. and Gagliardi, L.. High-performance liquid chromatography analysis of anti-inflammatory pharmaceuticals with ultraviolet and electro spray-mass spectrometry detection in suspected counterfeit homeopathic medicinal products. *J. Pharm. Biomed. Anal*. 2007;43: 1221-1227.

12.Diptish Ku. Nayak, Vankar Kaushik Kumar, Arabinda Patnaik., Simultaneous Estimation of Rabeprazole Sodium and Diclofenac Sodium by Rp-Hplc Method in Combined Tablet Dosage Form**.** *International Journal of Pharm. Tech Research*. 2010; 2(2): 1488-1492.

13.Nayak Kumar D.V. and Patnaik A., Recent advances in Parkinson disease. *International Journal of Pharm. Tech. Research.*2010;2:1488.

14.Cakirer, O., Kilic, E., Atakol, O. and Kenar, A. The non-aqueous titrimetric assay of the selected anti-inflammatory agents using tetra-n-butylammoniumhydroxide as titrant. *J. Pharm. Biomed. Anal*. 1999;20:19-26.

15. Shamsipur, M., Jalali, F., Ershad, S. Preparation of a diclofenac potentiometric sensor and its application to pharmaceutical analysis and to drug recovery from biological fluids. *J. Pharm. Biomed. Anal*. 2005;37:943-947.

16. Hassan, S. S. M., Mahmoud, W. H., Elmosallamy, M.A. F. and Almarzooqui, M. H. Iron(II)-phthalocyanineas a novel recognition sensor for selective potentiometric determination of diclofenac and warfarin drugs. *J. Pharm.Biomed. Anal*. 2005;39: 315-321.

17.The Official Compendia of Standards. The United States Pharmacopoeia. ,29 th edn.USP convention Inc, Rockville;2006:683.

18.British Pharmacopoeia, International edn. HMSO publication, London 2007;1:469.

19. Xu, M.T., Chen, L.F., Song, J.F. Polarographic behaviors of diclofenac sodium in the presence of dissolved oxygen and its analytical application. *Anal. Biochem*. 2004; 329: 21-27.

20.B.Gowramma, S. Rajan, S. Muralidharan, S. N. Meyyanathan and B. Suresh. A validated RP-HPLC method for simultaneous estimation of paracetamol and diclofenac potassium in pharmaceutical formulation. *International Journal of ChemTech Research*.2010;2(1):676-680.

21.Sznitowska M. and Stokrockam. Determination of diclofenac released from suppositories using UV spectrophotometry, spectra derivative spectrophotometry and HPLC . *Polish Pharmaceutical Society*. 2007; 63 (5): 401-405.