OPEN ACCESS

Pharmaceutical Communications
DOI: 10.55627/pharma.002.02.0000

Research Article

A Sensitive and Specific Analytical Method for Determination of Diclofenac Sodium Using High-Performance Liquid Chromatography

Muhammad Akhlaq[1], Humera Saeed[1], Rukhshanda Habib[2]

[1]Department of Pharmacy, Hazara University, Mansehra, Pakistan
[2]Faculty of Pharmacy, Gomal University, Dera Ismail Khan, Pakistan
***Correspondence**: akhlaqhayat@hotmail.com

Abstract

Diclofenac Sodium (DCL-Na) is a widely prescribed nonsteroidal anti-inflammatory drug (NSAID) used to treat conditions such as rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, and gout. This study aims to develop and validate a simple, precise, and accurate high-performance liquid chromatography (HPLC) method for quantifying DCL-Na in pharmaceutical formulations. The method utilizes a Supelcosil C-18 column with a mobile phase consisting of potassium dihydrogen phosphate solution and acetonitrile. The method validation follows International Conference on Harmonization (ICH) guidelines, evaluating precision, accuracy, specificity, linearity, and range parameters. The developed method demonstrated excellent linearity with an R^2 value of 0.9996 over a concentration range of 5-50 µg/ml, and the limits of detection (LOD) and quantification (LOQ) were found to be 6.634 µg/ml and 22.114 µg/ml, respectively. Intraday and interday precision tests showed relative standard deviations (% RSD) well within acceptable ranges. The method's practical applicability was confirmed through the analysis of controlled-release tablets, which showed a release of approximately 80% of DCL-Na after 20 hours. This validated HPLC method is suitable for routine quality control of DCL-Na in pharmaceutical products.

Keywords: Analytical Method, Diclofenac Sodium, HPLC-UV, Practical Application, Validation

1. Introduction

In routine clinical practice, physicians prescribe Diclofenac Sodium (DCL-Na), a widely used nonsteroidal anti-inflammatory drug (NSAID), as a first-line treatment for patients suffering from rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, and gout (Wortmann 2005, Cannon et al. 2006). The Current Good Manufacturing Practice (cGMP) requires that test methods used to analyze pharmaceutical products meet established accuracy and reliability standards. The above parameters are critical for product quality, safety, and efficacy. An accurate and precise analytical method can be used effectively. The method must first be validated to ensure its suitability for the intended application. The quality guidelines include precision, accuracy, specificity, linearity, and range to ensure reliability. Additionally, the LOQ (limit of quantification) and LOD (limit of detection) could be calculated (Stokvis, Rosing, and Beijnen 2005, Vial and Jardy 1999). The chromatography was preferred on a reversed-phase supelcosil LC-18, 5µm, 150 X 46mm column, with a mobile phase composed of methanol:acetonirile:0.02M sodium acetate buffer with pH 7 (25:20:55) (Akhter 2013). Various researchers have previously described diclofenac sodium determinations in biological fluids using HPLC (High-performance liquid chromatography) in analytical profiles. Similarly, Spherisorb RP-C-8 column (5 µm) and Acetonitrile/water (50:50 v/v, pH 3.3 using glacial acetic acid), mobile phase at 280nm, were used to determine the drug in plasma. Diclofenac sodium

in urine was determined using Nucleosil C-18 (10μm) and methanol-acetronitrile-0.02M acetate buffer pH 7 (NaCl 0.02M; 5:18:77) mobile phase (Abdel-Hamid, Novotny, and Hamza 2001). Another study used a Shim-pack GLC-CN 5μm column (150 x 4mm) and Spectra P 2000 HPLC system to detect DCL-Na. The mobile phase was prepared with acetonitrile ammonium acetate solution (20 mM) 5:1 v/v, pH 7.4, and flow rate was adjusted at 1ml/min (Fawcett, Morgan, and Woods 1997). Except for a few pharmacokinetic studies, no simple and rapid chromatographic method for quantifying DCL-Na has been published. The current study describes an accurate, simple, specific, precise, rapid, and authenticated HPLC method for quantitatively assessing DCL-Na. The method could be used to analyze DCL-Na in routine quality control laboratories for pure drugs and their dosage forms in the pharmaceutical industry.

2. Materials & Methods

Wilshire Pharmaceuticals in Lahore, Pakistan generously donated diclofenac sodium and diclotab® 25 mg. Monobasic potassium dihydrogen phosphate, HPLC grade water, and acetonitrile were purchased from Merck, Germany. The HPLC system is an Agilent 1200, Agilent Corporation, Germany, with ChemStation® software (Agilent Corporation, Germany). Milli-Q water was obtained from MilliQ, Millipore, USA, and beakers, flasks, and test tubes were obtained from the local market. The basic medical sciences lab at Gomal University's Faculty of Pharmacy supplied drug-free plasma.

2.1. HPLC-Method Development

A method described by Fawcett with slight modifications was used to perform this experiment (Shah et al. 1992). The High-Performance Liquid Chromatographic System used in this study was the Agilent-1200, manufactured by Agilent Corporation in Germany. It was equipped with a photodiode array (PDA-Detector), an automatic sampler, and a quaternary pump. Data acquisition was performed using ChemStation software ®, developed by Agilent in Germany. The separation process was conducted utilizing a Supelcosil C-18 column with dimensions of 5 μm for particle size and 4.6 mm×150 mm for column dimensions. The separation was carried out at a temperature of 25°C. The mobile phase was prepared by mixing 70 parts of a potassium dihydrogen phosphate solution (40 mM) with a pH of 4 with 30 parts of acetonitrile. The flow rate of 0.85 mL/minute was maintained at a constant level. The syringe was washed using a fixed volume of acetonitrile-water solution (70/30 v/v), specifically 5 μl. A wavelength of 276 nm was employed to identify the peak and assess the purity of DCL-Na.

2.2. Validated HPLC Analysis Methods

The developed method has been verified in accordance with the International Conference on Harmonization (ICH) guidelines (Marszałł et al. 2005).

The 10 mg of diclofenac sodium (DCL-Na) was precisely weighed. A volumetric flask was filled with 10 mL of distilled water (Milli-Q, Millipore, USA). The drug was then dissolved in water, forming a stock solution. Following that, a series of dilutions were made from the original solution using a 70:30 ratio of acetonitrile to water (v/v). These dilutions were used to produce a standard curve with concentrations ranging from 5 to 50 μg/ml.

2.3. Precision & Accuracy

The accuracy was evaluated by performing recovery assays, where predetermined amounts of standards (8, 18, and 40 ug/ml) were introduced into the sample. The solution was injected three times (Guideline, 2005, Landim et al., 2013, Seo et al., 2016). While, to evaluate the precision of this method within a 24-hour period, the sample is injected three times per day. The inter-day precision was evaluated by analyzing samples on multiple days and by engaging a second analyst.

Table 1: Reverse predicted concentrations, % recovery, and regression coefficient (R^2)

Set number	Nominal concentration (µg/ml)								R^2	Slope	Intercept
	5	10	15	20	25	30	35	50			
1	5.34	9.68	14.50	19.91	25.44	30.95	34.13	50.00	0.9984	13.481	9.5682
2	4.95	10.11	14.96	19.43	25.15	30.26	35.48	49.65	0.9995	13.58	5.127
3	5.07	10.31	15.35	19.77	25.14	29.55	33.93	50.86	0.9984	13.210	3.0877
4	5.29	10.02	14.48	19.71	25.67	29.14	35.88	49.80	0.9983	13.324	7.0479
5	5.21	9.77	14.51	20.22	26.46	29.22	36.00	50.32	0.9975	13.280	7.6631
Mean	5.17	9.98	14.76	19.80	25.57	29.83	34.75	50.13	0.9996	13.375	6.4988
% RSD	1.59	4.39	3.84	0.73	1.60	3.40	3.18	0.81			
% Recovery	103.53	99.80	98.40	99.00	102.00	99.43	99.28	100.26			

2.4. Limit of Detection (LOD) And Limit Of Quantification (LOQ)

LOD (Detection limit) is the lowest detectable amount of analyte in a sample while The limit of quantitation (LOQ) (quantitative limit) is the lowest amount of analyte in a sample that can be quantified with sufficient accuracy and precision. Calibration curve is formed to calculate LOD and LOQ limits. According to ICH Guidelines,the following equations are used for the determination of LOD and LOQ respectively.

$$LOD = \frac{3\sigma}{S} \text{.....} Equation: 1$$

$$LOQ = \frac{10\sigma}{S} \text{.....} Equation: 2$$

Where,

σ=intersection and standard deviation,S = slope of the calibration curve.

2.5. Specificity (Determination of Maximum Purity)

The PDA-Detector, equipped with the Agilent ChemStation software, facilitated the acquisition of UV-Spectra online data for the drug DCL-Na during its maximum elution. The data was collected within the wavelength range of 190-450nm (Sinha et al. 2007, ICH 2005). The detector employed in the HPLC system provides additional evidence regarding the distribution of the sample. UV spectra were collected at five distinct positions along the peak of diclofenac sodium: two points prior to the highest point (apex) of the peak, two points after the apex (front of tail), and one point at the apex itself. The highest level of purity was achieved by analyzing the similarity of the UV spectra obtained at the five points. If any adulterated or degraded product coelutes with the drug diclofenac sodium, the five UV spectra obtained from the peaks will be distinct. Throughout the experiment, all diclofenac sodium drug samples underwent maximum purity analysis.

2.6. Practical Applicability

Two specific DCL-Na controlled release tablets were tested for their release properties using the PharmaTest Dissolution Apparatus (basket method) with 900ml of phosphate buffer solution (pH 7.4) at a constant temperature of 37±2°C. Various samples were extracted and examined at specific time intervals (0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 18, and 24 hours) using the previously mentioned HPLC method to verify the effectiveness of the developed method in practical settings.

3. Results & Discussion

3.1. HPLC Method Development

High-performance liquid chromatography (HPLC) is a modern and flexible separation method frequently used to separate, identify, and quantify substances in order to obtain their

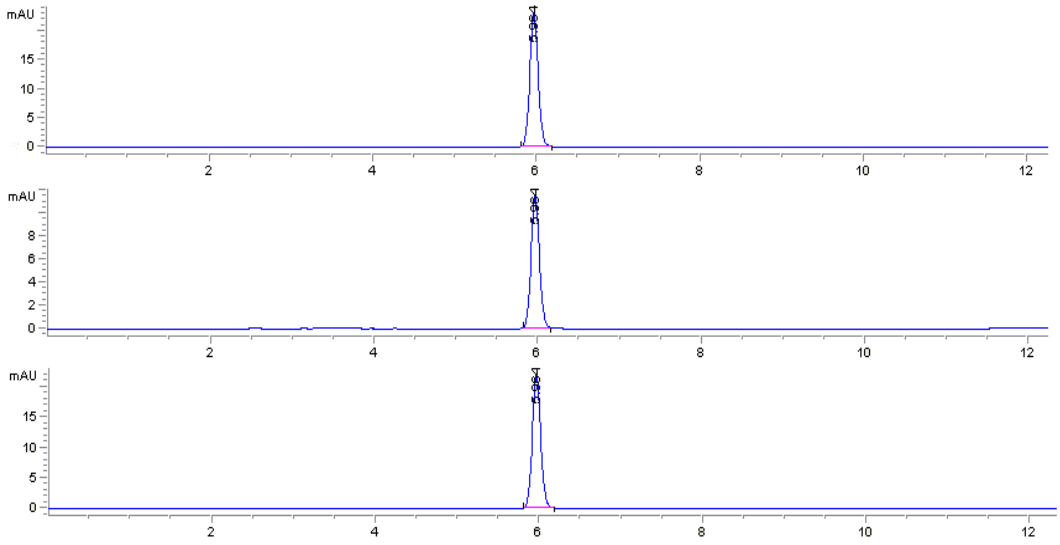

Figure 1. Representative HPLC chromatograms at three different concentrations using mobile phase

Table 1. Precision and accuracy data of the QC samples (Results were expressed as mean values, n = 5)

Parameters	Nominal concentration (µg/ml)					
	Intra-day			Inter-day		
	8	25	45	8	25	45
Mean	7.668	25.611	44.717	7.926	25.001	44.888
% RSD	0.0006	0.0003	0.0002	0.0005	0.0006	0.0053
% Recovery	95.850	102.444	99.371	99.075	100.004	99.751

chemical profiles (Sarker and Nahar, 2015). The parameters for analyzing DCL-Na were assessed by modifying their analytical parameters. The objective is to determine the optimal conditions for analyzing compounds. The process of optimization involved modifying various parameters until the most favorable conditions were attained. The system's adequacy parameters were found to be within an acceptable range. Each sample solution had an analysis run time of 8 minutes. The retention time of DCL-Na under study conditions was 5.984 minutes, which was well defined by the formulation excipients (Figure 1). The parameters listed below were evaluated, estimated, and evaluated in accordance with the ICH guidelines (Sinha et al. 2007, Wiberg et al. 2004).A proposed alternative method aims to replace the current methodology used for comparing laboratory data and implement it in practical applications, taking into account all the potential advantages and disadvantages (Patil, 2017).

3.2. Linearity

The study revealed that there was a linear relationship between the peak area of the standard calibration curve and the concentration within the range of 5-50 mg/ml. The linearity coefficient, R2, was greater than 0.999. The recovery percentage (%) with a ±% relative standard deviation (DER) ranged from 98.40 ± 3.84 to 103.53 ± 1.59 (Table 1).

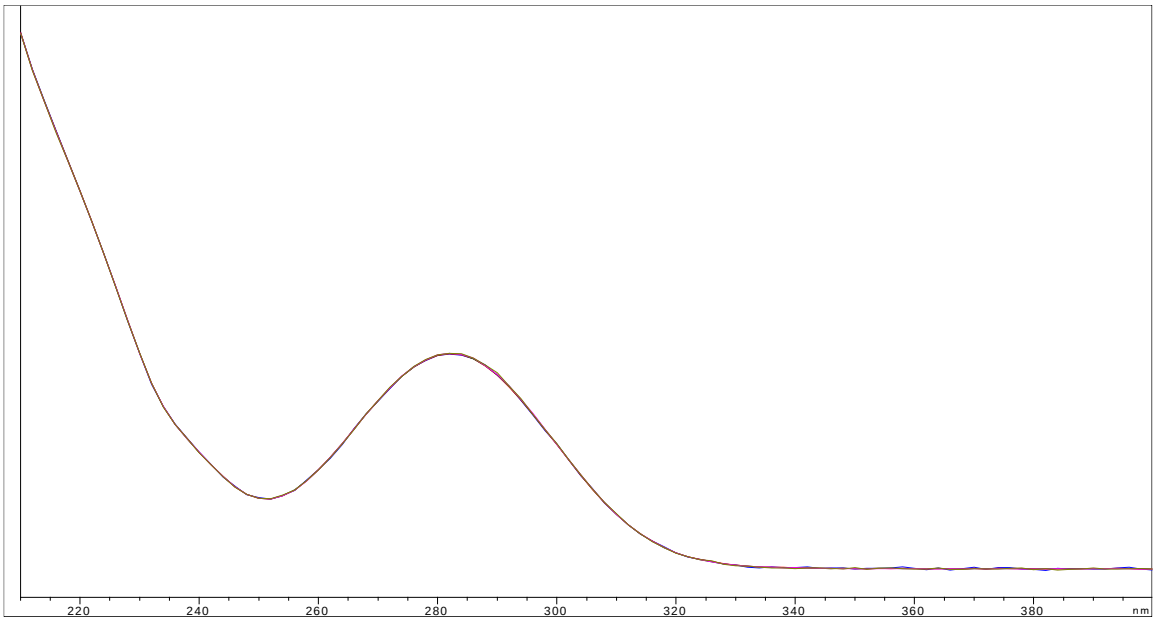

Figure 1. UV- UV-absorption spectra of the DCL-Na peak from a standard solution

Statistical computations were conducted at a 95% confidence level. The linear regression equation derived from the proposed method was discovered. The slope value reported, without an intercept on the y-axis, along with its 95% confidence limits, indicates that the calibration point of the drug solution does not deviate from the origin. This is supported by the fact that the value falls within the confidence limit.

3.3. Limit Of Detection and Quantification (LOD and LOQ)

The detection and quantification limits were determined to be 6.634 µg/ml and 22.114 µg/ml, respectively.

3.4. Precision & Accuracy

The precision and accuracy of the method are described by the values of the relative standard deviation (% RSD) and the percentage of recovery of the controlled quality samples, as shown in table 2. The study revealed that the samples exhibited intraday precision ranging from 0.0005% to 0.0053% and interday precision ranging from 0.0002% to 0.0006%. The results meet the acceptance criteria outlined in the ICH guidelines. The study ensured the purity of diclofenac sodium peaks by utilizing the PDA-Detector and Agilent-ChemStation software. This was achieved by analyzing the UV spectra at four specific time points within the diclofenac sodium peak, from the beginning to the end of the elution process. Figure 2 displays the elution spectra of Diclofenac Sodium obtained during the execution time. It was found that the determined points consist of two points with increasing slope (one starting point and one ending point) specifically for assessing purity, indicating a single component and a pure peak. The calibration curve, depicted in Figure 3, exhibited exceptional linearity with an R2 value of 0.9996.

3.5. Practical Applicability

The studies on DCL-Na-CR tablets revealed that around 80% of DCL-Na was released after 20 hours. This was determined using the HPLC method mentioned earlier, with a wavelength of 276 nm and a retention time of 5.984 min. Thus, it can be noted that investigations on the release of controlled-release matrix tablets can validate the suitability of the HPLC method devised for the drug DCL-Na.

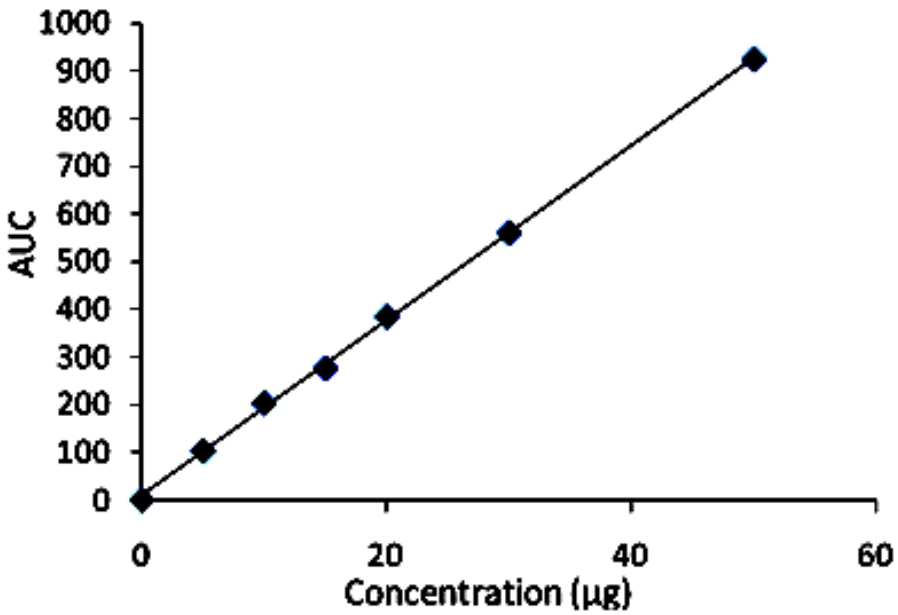

Figure 3. Mean standard HPLC calibration curve for DCL-Na

4. Conclusion

The study effectively created and confirmed a strong HPLC technique for accurately measuring the amount of diclofenac sodium in pharmaceutical products. The method demonstrates exceptional precision, accuracy, and specificity, in accordance with the guidelines set by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). The validation parameters, such as linearity, limit of detection (LOD), limit of quantification (LOQ), and recovery, demonstrate the method's reliability for routine quality control applications. Furthermore, the method's practical applicability was demonstrated by conducting dissolution studies on controlled-release tablets, which confirmed its effectiveness in real-world pharmaceutical analysis. This validated method provides a valuable tool for guaranteeing the quality and effectiveness of pharmaceutical products containing DCL-Na in the pharmaceutical industry.

Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

Funding

There was no specific funding available for this project.

Study Approval

There are no human subjects involved so, this study requires no institutional or ethical review board approval.

Consent Forms

NA.

Authors Contributions

MA was responsible for conducting the study by performing lab analysis, conceptualization, methodology, validation, formal analysis, investigation, and writing the original draft. HS was responsible for writing – reviewing & editing. RH was responsible for conceptualization,

methodology, supervision, writing – review & editing, and project administration.

Acknowledgments
None

References

Abdel-Hamid, Mohammed E, Ladislav Novotny, and Hoda Hamza. 2001. "Determination of diclofenac sodium, flufenamic acid, indomethacin and ketoprofen by LC-APCI-MS." *Journal of pharmaceutical and biomedical analysis* no. 24 (4):587-594.

Akhter, Ruplay. 2013. *EXPERIMENTAL DICLOFENAC TOXICITY IN BROILERS*.

Cannon, Christopher P, Sean P Curtis, Garret A FitzGerald, Henry Krum, Amarjot Kaur, James A Bolognese, Alise S Reicin, Claire Bombardier, Michael E Weinblatt, and Désirée Van Der Heijde. 2006. "Cardiovascular outcomes with etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) programme: a randomised comparison." *The Lancet* no. 368 (9549):1771-1781.

Fawcett, J Paul, Nicola C Morgan, and David J Woods. 1997. "Formulation and stability of naltrexone oral liquid for rapid withdrawal from methadone." *Annals of Pharmacotherapy* no. 31 (11):1291-1295.

ICH. 2005. "ICH Harmonised Tripartite Guideline (2005) Validation of Analytical Procedures: Text and Methodology Q2(R1). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, 1-13.".

Marszałł, M. L., A. Lebiedzińska, W. Czarnowski, and P. Szefer. 2005. "High-performance liquid chromatography method for the simultaneous determination of thiamine hydrochloride, pyridoxine hydrochloride and cyanocobalamin in pharmaceutical formulations using coulometric electrochemical and ultraviolet detection." *J Chromatogr A* no. 1094 (1-2):91-8. doi: 10.1016/j.chroma.2005.07.091.

Shah, Vinod P., Kamal K. Midha, Shrikant V. Dighe, Iain J. McGilveray, Jerome P. Skelly, Avraham Yacobi, Thomas Layloff, Ct Viswanathan, C.Edgar Cook, R. D. McDowall, Kenneth A. Pittman, Sidney A. Spector, Kenneth Stephen Albert, Sanford M. Bolton, Michael R. Dobrinska, William H. Doub, Michel F Eichelbaum, John W. A. Findlay, Keith D. Gallicano, William A. Garland, Dwight J. Hardy, James D. Hulse, H. Thomas Karnes, Ron Lange, William David Mason, Gordon Mckay, Eric D. Ormsby, James G. Overpeck, H.D. Plattenberg, Gerald K. Shiu, Daniel S. Sitar, Fritz Sorgel, James T. Stewart, and Lee Yuh. 1992. "Analytical Methods Validation: Bioavailability, Bioequivalence and Pharmacokinetic Studies." *Pharmaceutical Research* no. 9:588-592.

Sinha, VR, Monika, A Trehan, Manoj Kumar, S Singh, and JR Bhinge. 2007. "Stress studies on acyclovir." *Journal of chromatographic science* no. 45 (6):319-324.

Stokvis, Ellen, Hilde Rosing, and Jos H Beijnen. 2005. "Liquid chromatography-mass spectrometry for the quantitative bioanalysis of anticancer drugs." *Mass spectrometry reviews* no. 24 (6):887-917.

Vial, Jérôme, and Alain Jardy. 1999. "Experimental comparison of the different approaches to estimate LOD and LOQ of an HPLC method." *Analytical Chemistry* no. 71 (14):2672-2677.

Wiberg, Kent, Mattias Andersson, Anders Hagman, and Sven P Jacobsson. 2004. "Peak purity determination with principal component analysis of high-performance liquid chromatography–diode array

detection data." *Journal of Chromatography A* no. 1029 (1-2):13-20.
Wortmann, Robert L. 2005. "Recent advances in the management of gout and hyperuricemia." *Current opinion in rheumatology* no. 17 (3):319-324.