Brazilian Journal of Analytical Chemistry
2023, Volume 10, Issue 39, pp 70-79
doi: 10.30744/brjac.2179-3425.AR-35-2022

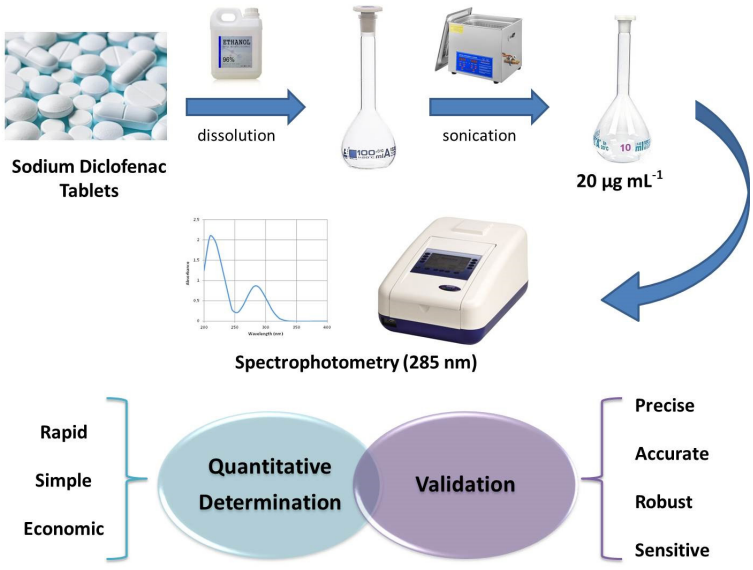
BrJAC

ARTICLE

Development and Validation of a Simple Spectrophotometric Method for Quantitative Determination of Sodium Diclofenac in Modified-Release Tablets

Razan Shoujaa Alkassab*, **Yumen Hilal**, **Amin Swed**

Faculty of Pharmacy, Al-Baath University, P.O. Box 77, Damascus Highway, Homs, Syria



In this study, a rapid, simple, cost-effective and accurate spectrophotometric method was developed for the determination of sodium diclofenac in modified release tablets using ethanol 96% as an available and non-toxic solvent. Sodium diclofenac standard solution was scanned under UV (200-400 nm) in a 1 cm quartz cell to determine the maximum absorption wavelength which was 285 nm. This method was validated in accordance with the requirements of the International Conference on Harmonization (ICH), and the calibration curve showed linearity in the studied concentration range (5-30 µg mL^{-1}) with correlation coefficient R^2 = 0.9993. The relative standard deviation of the accuracy studies was within the acceptable range (<2%). This method also achieved an excellent recovery ratio (Mean recovery ± S.D. = 100.44% ± 0.81) with high sensitivity (limit of detection 1.10 µg mL^{-1} and quantitation limit of 3.34 µg mL^{-1}). The developed method applied successfully to determine sodium diclofenac in four commercial pharmaceuticals products (A, B, C and D) marketed locally as modified-release tablets. The product C showed the highest assay value 106% and product B showed the lowest value 98%. Therefore, we recommend using this method to quantitatively determine sodium diclofenac in pharmaceutical dosage forms.

Keywords: sodium diclofenac, UV spectrophotometry, method validation, modified-release tablets, quantitative determination

Cite: Alkassab, S. R.; Hilal, Y.; Swed, A. Development and Validation of a Simple Spectrophotometric Method for Quantitative Determination of Sodium Diclofenac in Modified-Release Tablets. *Braz. J. Anal. Chem.*, 2023, *10* (39), pp 70-79. http://dx.doi.org/10.30744/brjac.2179-3425.AR-35-2022

Submitted 13 May 2022, Resubmitted 06 July 2022, Accepted 06 August 2022, Available online 31 August 2022.

INTRODUCTION

Sodium diclofenac (SD) is chemically sodium salt of [o-(2,6-dichloroaniline)phenyl] acetate.[1] Its molecular formula is $C_{14}H_{10}C_{l2}NNaO_2$ (Figure 1), and the molecular weight is 318.1. It is sparingly soluble in water and soluble in ethanol (96%).[2] SD is a non-steroidal anti-inflammatory drug (NSAID)[3] that inhibits cyclooxygenase enzymes that prevent the conversion of arachidonic acid into prostaglandins. It is widely used to manage pain, fever and inflammation associated with many clinical conditions, such as degenerative joint diseases, acute and chronic musculoskeletal disorders, sports injuries and post-surgical analgesia in humans and animals.[4,5] It also exhibits anticancer effects.[6]

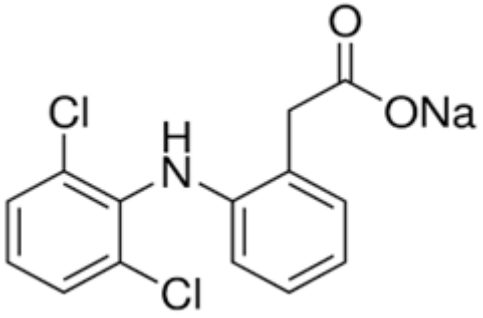


Figure 1. The chemical structure of sodium diclofenac.

Nausea, gastritis, skin redness and headache are the common side effects of this drug. As well, it has a short half-life of 1-2 hours, so it was designed as a modified-release tablet to decrease the frequency of drug administration, reduce unpleasant side effects and to minimize fluctuations in blood drug levels, thus improving patient compliance.[7,8]

Several different analytical methods have been recorded for the estimating of SD in dosage forms and bulk, like spectrophotometry,[9,10] voltammetry utilizing liquid modified nanotubes,[11] gas chromatography-mass spectrometry,[11] and high-performance liquid chromatography (HPLC), which is the foremost common process for determining SD in dosage forms and biological fluids.[12-14]

SD has also been determined by creating selective membrane electrodes for SD by mixing different plasticizers.[15] Despite the accuracy of previous methods, some require sophisticated equipment and solvents that may be toxic to the environment and some take a relatively long time to prepare the sample.

In order to overcome the difficulties of previous techniques, save time and avoid toxic organic solvents, this research aimed to develop a fast, simple, selective, sensitive and cost-effective spectrophotometric method, which was validated in accordance with the guidelines of the International Conference on Harmonization ICH Q1(R2).[1,16]

MATERIALS AND METHODS

Instruments

Spectrophotometric determination of SD was performed using a 7315 UV-visible spectrophotometer (Jenway Scientific Equipment, UK) fitted with a 1 cm quartz cuvette and xenon lamp. Analysis results were acquired on a Windows computer system using a Jenway 73 Series. A sensitive electronic balance (model Sartorius ED224-S, Germany) was used to accurately weigh the materials, an ultrasonic bath (model Digital pro MC-PS-06A, China) and a magnetic hotplate stirrer (model AKMLAB SH-4C, China) were also used for homogenization.

Chemicals

SD standard powder (99.87%) from PHARMCHEM Pharmaceutical Ltd, Haryana, India, and ethanol 96% (Merck, Germany) were used in this study. Four brands of modified SD tablets manufactured and

marketed in Syria were randomly obtained and coded as A, B, C and D. The labeled active ingredient of SD was 100 mg packaged in blister.

Preparation of standard stock solution

The standard stock solutions of SD were prepared by weighing 100 mg of standard powder and dissolved in 50 mL ethanol 96% in a 100 mL volumetric flask. Volume was made up to the mark with ethanol 96% to get a solution at a concentration of 1000 µg mL^{-1}. The flask was sonicated for 2 min to give clear solution. Then, 10 mL of this solution was diluted to 100 mL with ethanol 96% to give a concentration of 100 µg mL^{-1}.

Determination of absorption maxima

2 mL of the SD standard solution was transferred to a 10 mL volumetric flask and the volume was made up to the mark with 96% ethanol to obtain a concentration of 20 µg mL^{-1}. The resulting solution was scanned within the UV range 200-400 nm, with ethanol 96% as blank. The spectrum displayed the absorbance maximum of SD at 285 nm (Figure 2).

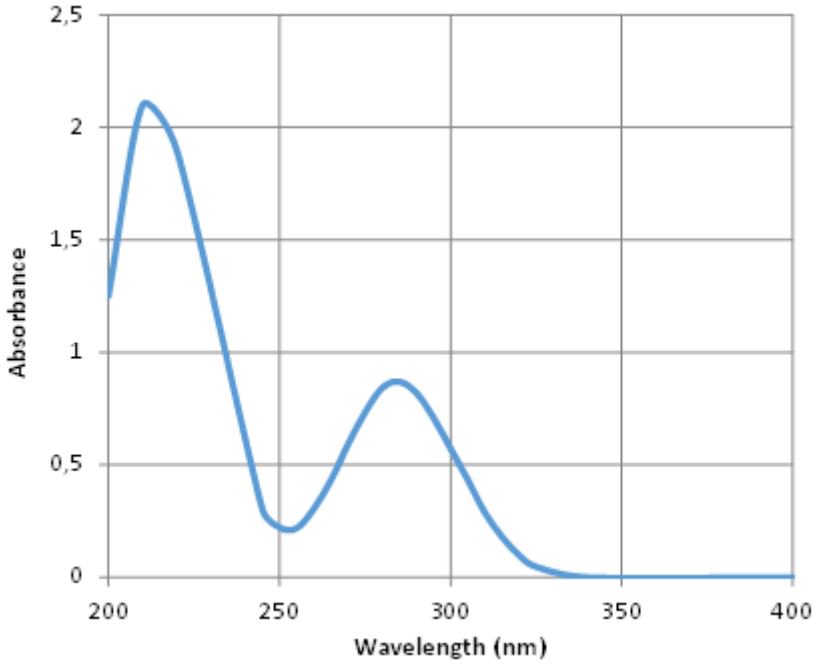


Figure 2. UV spectrum of sodium diclofenac (200-400 nm).

Validation of diclofenac UV-spectrophotometric assay

The developed method was validated by evaluating its linearity, accuracy, precision, sensitivity and robustness according to the Q2 (R1) Recommendations of the (ICH) and USP Validation Guidelines.[1,16–18]

Linearity

Linearity is the capability of a method to deliver results which are directly proportional to the concentration of the analyte. Linearity was specified by pipetting out 0.5, 1, 1.5, 2, 2.5 and 3 mL from the stock solution (100 µg mL^{-1}) into a 10 mL volumetric flask and diluted to the mark with ethanol 96%. The final concentrations of these solutions were 5, 10, 15, 20, 25, 30 µg mL^{-1} respectively. The absorbance of each solution was measured at wavelength 285 nm and ethanol 96% was used as a blank. The observations were recorded and graphically presented.[14,16,17]

Precision

The precision of the analytical process expresses the relative agreement (degree of dispersion) between a set of measurements taken from numerous samples of the same homogeneous sample under the given conditions. In this method, precision was studied as interday and intraday variations. Interday precision (intermediate precision) was determined by analyzing the SD solution 20 µg mL^{-1} for 3 days during a period of one week. Intraday precision (repeatability) was determined similarly, but the analysis was performed five times on the same day. The absorbance of each solution was measured and recorded as the relative standard deviation to obtain the variance.[18,19]

Accuracy

The accuracy of an analytical method expresses the proximity between the concentration found and the expected concentration, which was determined by preparing solutions of three concentrations 15, 20, 25 µg mL^{-1}. The absorbance of the solutions was measured at 285 nm and the results were expressed in terms of recoveries (%). Mean of actual concentration ± (RSD%) and standard deviation were calculated.[16]

Robustness

The robustness of an analytical method could be a degree of its capacity to stay not influenced by little, but deliberate changes in method parameters and provides a sign of its reliabilityduring normal use. In this study, the absorption maxima was decreased and increased 2 nm with the solution of 20 µg mL^{-1}, for 5 times. The RSD% was calculated.[20]

Limit of Detection (LOD)

It is the smallest amount of analyte which can be identified and unnecessarily quantified. It has been calculated by Equation 1.[17]

$$LOD = 3.3 \times S.D./Slope \qquad \text{Equation 1}$$

where: S.D. = Standard Deviation and Slope = the slope of the calibration curve

Limit of Quantitation (LOQ)

It is the lowest concentration of the sample that could be determined with acceptable accuracy and precision. It was calculated by Equation 2.[17]

$$LOQ = 10 \times S.D./Slope \qquad \text{Equation 2}$$

where: S.D. = Standard Deviation and Slope = the slope of the calibration curve

Analysis of a marketed dosage forms

Standard solution

100 mg of SD standard was added to a 100 mL volumetric flask containing 50 mL ethanol 96%. The flask was sonicated for 2 min to give a clear solution and the volume was made up to mark with ethanol 96%. 2 mL of this solution was diluted to 10 mL with ethanol 96% to give a standard solution of 20 µg mL^{-1}.

Sample Preparation

Twenty tablets of four different modified-release SD tablets were weighed accurately and powdered using a mortar and pestle. After calculating the average weight of one tablet, powder weighted as the equivalent of 100 mg SD was added to a 100 mL volumetric flask containing 50 mL ethanol 96%. The flask was sonicated for 2 min to give a clear solution and volume was made up to mark with ethanol 96%. From this, 2 mL was taken and diluted to 10 mL with ethanol 96% that gives 20 µg mL^{-1} solution and the absorbance of the solution was measured at 285 nm.

Procedure

The prepared standard and sample solutions were scanned in a spectrophotometer at 285 nm in 1 cm quartz cell, using ethanol 96% as a blank solution. The concentrations were calculated by substituting the absorbance values in the linear regression equation.[20,21]

Statistical analysis

Microsoft Excel 2010 was used to process the data, where the regression equation and the correlation coefficient of linearity were calculated, moreover the mean, standard deviation (S.D.) and relative standard deviation (RSD) that were calculated to check the precision, accuracy and robustness of the method.

RESULTS AND DISCUSSION

The analytical technique in this work based on ultraviolet spectroscopy was developed for the estimation of SD in its pharmaceutical dosage forms. It is a low-cost process and requires simple equipment compared to the chromatography methods mentioned in the United States and British Pharmacopoeia.[2,18] Ethanol 96% was chosen as a solvent, as it is a good solvent for SD[2] in addition to its availability and non-toxicity compared to other organic solvents. This method did not require a long time to complete the analysis which took 20 minutes to estimate and quantify SD compared to the HPLC method in USP 41 NF 36[1] which took about 50-60 minutes, thus a larger number of samples can be analyzed in a relatively large time.

Method validation

Linearity

The linear regression information for the calibration curves showed an excellent linear relationship over the concentration 5-30 µg mL^{-1} for SD. The regression equation was found to be Y= 0.0399X + 0.0247 (Equation 3) where the correlation coefficient R^2 = 0.9993. (Figure 3, Table I).

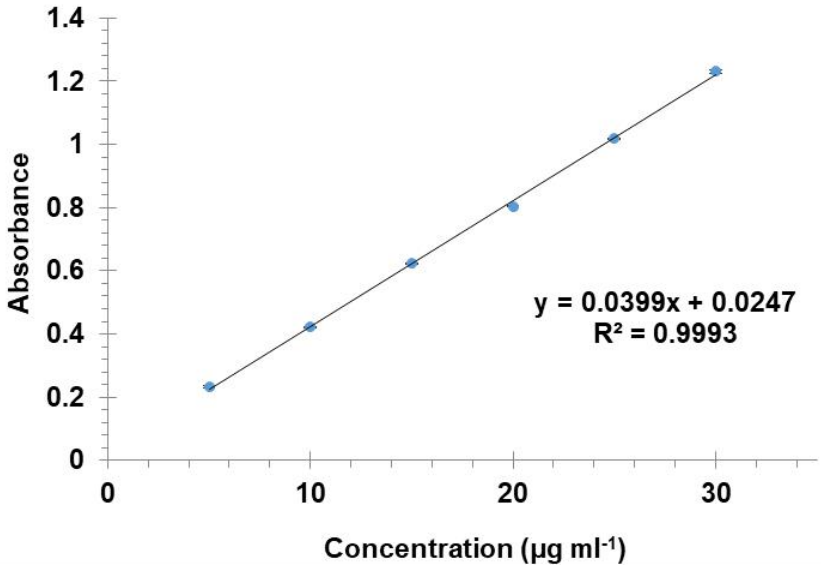


Figure 3. Calibration curve of sodium diclofenac.

Table I. Calibration curve data for sodium diclofenac

Sample No	Drug concentration (μg mL^{-1})	Mean Absorbance ± S.D. (n = 5)	RSD %
1	5	0.229 ± 0.002	0.79
2	10	0.422 ± 0.002	0.37
3	15	0.623 ± 0.002	0.37
4	20	0.804 ± 0.002	0.26
5	25	1.019 ± 0.002	0.18
6	30	1.231 ± 0.006	0.48

Precision

Precision studies have shown good results meeting ICH requirements for intraday and interday precision. The RSD% value for intraday precision absorbance was 0.37%, while it was 0.72% for interday precision as mentioned in Table II. Thus, the RSD% values match the acceptance criteria (less than 2%), which confirms that the developed method has good precision.

Table II. Results of the precision studies

Sample No	Absorbance at 285 nm	
	Intraday Precision (Repeatability)	Interday Precision (intermediate precision)
1	0.802	0.807
2	0.796	0.802
3	0.801	0.794
4	0.804	0.794
5	0.802	0.796
Mean Absorbance ± S.D. (n = 5)	0.801 ± 0.003	0.798 ± 0.006
RSD %	0.37	0.72

Accuracy

The accuracy of the studied process was confirmed by calculating the relative error, individual recovery and the mean recovery value of SD in its solutions, where the absorbance of nine solutions was measured at a wavelength of 285 nm belonging to three different concentrations (15, 20, 25 μg mL^{-1}) and three replicates for each concentration. The mean recovery was 100.44%, which corresponds the acceptance criteria (minimum of 98.0% and maximum of 102.0%) and the RSD% value for recovery was 0.80% as shown in Table III and this confirms the good accuracy of this method.

Table III. Accuracy results

Sample no	Accuracy level	Theoretical Concentration (µg mL^{-1})	Actual Concentration (µg mL^{-1})	Relative error	Recovery %
1	75%	15.00	14.894	-0.706	99.294
2	75%	15.00	15.207	1.380	101.380
3	75%	15.00	15.111	0.738	100.738
4	100%	20.00	20.134	0.671	100.671
5	100%	20.00	19.911	-0.447	99.553
6	100%	20.00	20.259	1.293	101.293
7	125%	25.00	24.848	-0.608	99.392
8	125%	25.00	25.216	0.863	100.863
9	125%	25.00	25.191	0.765	100.765
		Mean ± S.D.			100.44 ± 0.81
		RSD %			0.805

Robustness

The robustness of the developed method was examined by deliberately changing in the maximum absorption wavelength. The mean absorbance, relative standard deviation and standard deviation was calculated. The mean RSD% in this method was 0.601, that confirms the robustness of the method and the absence of significant changes in the absorbance values (Table IV).

Table IV. Robustness results

	Absorbance		
Concentration (µg mL^{-1})	**at 283 nm**	**at 285 nm**	**at 287 nm**
	0.802	0.809	0.801
	0.812	0.803	0.802
20 µg mL^{-1}	0.808	0.814	0.813
	0.804	0.808	0.809
	0.803	0.816	0.811
Mean Absorbance ± S.D. (n = 5)	0.806	0.810	0.807
S.D.	0.004	0.005	0.005
RSD %	0.50	0.64	0.67
mean RSD %		0.601	

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

The sensitivity of this method is ascertained by calculating the LOQ and LOD. Depending on the slope value = 0.0399 from Equation 3 and applying both Equations 1 and 2, the LOQ and LOD were calculated to be equal to 1.10 μg mL^{-1} and 3.34 μg mL^{-1}, respectively. These findings show that the method has good sensitivity.

Analysis of SD in the marketed dosage forms

The absorbance of the studied samples was recorded at the wavelength 285 nm. From the linear regression equation drug concentrations were calculated. The percentage of the amount of SD in its dosage forms ranged between 98% and 106% as clarified in Table V and Figure 4, which matches acceptance criteria (90 - 110% in USP 41 NF 36)[1]. As a result, this method could be used to quantitatively determine SD in its pharmaceutical dosage forms.

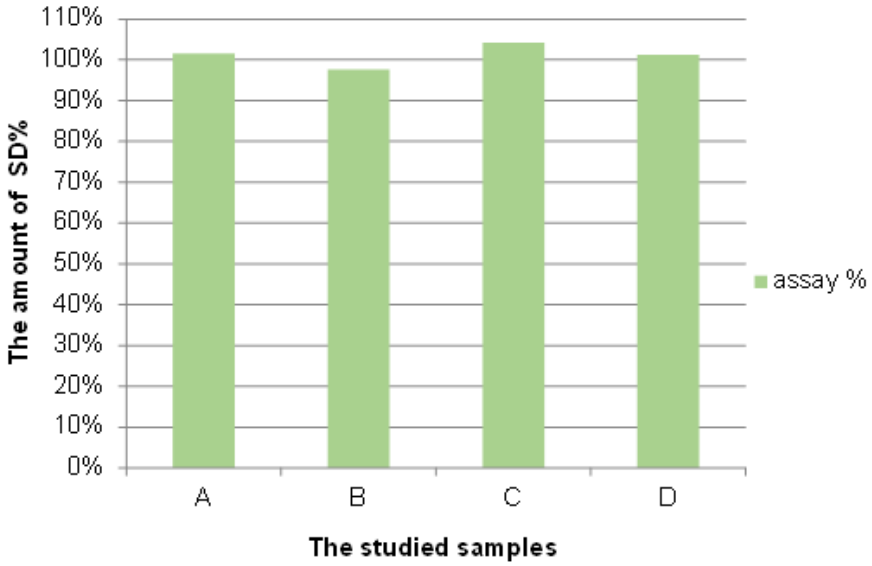


Figure 4. Assay of different brands of sodium diclofenac.

Table V. Results of sodium diclofenac determination in commercial formulation

Code	Dose (mg/tab)	Absorbance at 285 nm	Assay %
A	100	0.837	102%
B	100	0.806	98%
C	100	0.859	106%
D	100	0.835	101%

CONCLUSIONS

In this study, a simple spectrophotometric method based on UV spectroscopy has been developed in order to the estimation and assay of SD in the bulk and modified release tablets. The proposed method was validated and found to be a linear, accurate, reproducible, fast, simple and economical method, which does not require the sophisticated instruments and equipment used in chromatography method. Ethanol 96% was used as a solvent, which is available, non-toxic and low cost compared to other solvents. Therefore, it is recommended to use this method effectively to quantitatively determine sodium diclofenac in its pharmaceutical dosage forms for routine quality control.

Conflicts of interest

The authors declare that there is no financial conflict of interest.

Acknowledgments

The authors gratefully acknowledge ALMAERIFE Pharmaceutical industries (ALMA) and college of Pharmacy and its laboratories, Albaath University, Homs, Syria for their support.

REFERENCES

(1) The United States Pharmacopeia and National Formulary, USP 41–NF 36, **2018**.
(2) British Pharmacopoeia, Vol I, **2016**.
(3) Jahnavi, K.; Reddy, P. P.; Vasudha, B.; Narender, B. Non-Steroidal Anti-Inflammatory Drugs: An Overview. *J. Drug Deliv. Ther.* **2019**, *9* (1-s), 442–448. https://doi.org/10.22270/jddt.v9i1-s.2287
(4) Kołodziejska, J.; Kołodziejczyk, M. Diclofenac in the Treatment of Pain in Patients with Rheumatic Diseases. 2018, 174–183. https://doi.org/10.5114/reum.2018.76816
(5) Yang, L.; Li, J.; Li, Y.; Zhou, Y.; Wang, Z.; Zhang, D.; Liu, J.; Zhang, X. Diclofenac Impairs the Proliferation and Glucose Metabolism of Triple-negative Breast Cancer Cells by Targeting the c-Myc Pathway. *Exp. Ther. Med*. **2021**, *21* (6), 1–9. https://doi.org/10.3892/etm.2021.10016
(6) Yang, L.; Li, J.; Li, Y.; Zhou, Y.; Wang, Z.; Zhang, D.; Liu, J.; Zhang, X. Diclofenac Impairs the Proliferation and Glucose Metabolism of Triple-negative Breast Cancer Cells by Targeting the C-Myc Pathway. *Exp. Ther. Med.* **2021**, *21* (6), 1–9. https://doi.org/10.3892/etm.2021.10016
(7) Al Hanbali, O. A.; Hamed, R.; Arafat, M.; Bakkour, Y.; Al-Matubsi, H.; Mansour, R.; Al-Bataineh, Y.; Aldhoun, M.; Sarfraz, M.; Dardas, A. K. Y. Formulation and Evaluation of Diclofenac Controlled Release Matrix Tablets Made of HPMC and Poloxamer 188 Polymer: An Assessment on Mechanism of Drug Release. *Pak. J. Pharm. Sci.* **2018**, *31* (1), 345-351.
(8) Shahiwala, A.; Zarar, A. Pharmaceutical Product Lead Optimization for Better In vivo Bioequivalence Performance: A Case Study of Diclofenac Sodium Extended Release Matrix Tablets. *Curr. Drug Deliv.* **2018**, *15* (5), 705–715. https://doi.org/10.2174/1567201814666171120124551
(9) Mane, R. V.; Patel, K.; Sushmitha, G. S.; Vasantharaju, S. G. Development and Validation of Diclofenac Sodium in Tablets Using Simple UV Spectrophotometric Method. *Res. J. Pharm. Technol.* **2019**, *12* (2), 611–614. https://doi.org/10.5958/0974-360X.2019.00108.2
(10) Karnakar, N.; Ramana, H.; Amani, P.; Tharun, D. S.; Nagaraju, M.; Sharma, S. B. Analytical Method Development and Validation of Diclofenac Sodium by UV-Visible Spectroscopy Using AUC Method. *International Journal of Multidisciplinary Research and Development* **2020**, 7 (1), 20-24.
(11) Ensafi, A. A.; Izadi, M.; Karimi-Maleh, H. Sensitive voltammetric determination of diclofenac using room-temperature ionic liquid-modified carbon nanotubes paste electrode. *Ionics* **2013**, *19*, 137–144. https://doi.org/10.1007/s11581-012-0705-0
(12) Rosca, A. C.; Roncea, F. N.; Iancu, V.; Stoicescu, I.; Cazacincu, R.; Iancu, I.; Miresan, H. Development and Validation of HPLC-DAD Method for Diclofenac Sodium Assay from New Orodispersible Tablets with Co-Processed Excipients. *Rev. Chim.* **2020**, *71* (2), 335-342. https://doi.org/10.37358/RC.20.2.7934
(13) Cordery, S. F.; Pensado, A.; Chiu, W. S.; Shehab, M. Z.; Bunge, A. L.; Delgado-Charro, M. B.; Guy, R. H. Topical Bioavailability of Diclofenac from Locally-Acting, Dermatological Formulations. *Int. J. Pharm.* **2017**, *529* (1–2), 55–64. https://doi.org/10.1016/j.ijpharm.2017.06.063
(14) Alquadeib, B. T. Development and Validation of a New HPLC Analytical Method for the Determination of Diclofenac in Tablets. *Saudi Pharm. J.* **2019**, *27* (1), 66–70. https://doi.org/10.1016/j.jsps.2018.07.020
(15) Al-Bayati, Y. K.; Al-Safi, A. J. Synthesis and Characterization of a Molecularly Imprinted Polymer for Diclofenac Sodium Using (2-Vinylpyridine and 2-Hydroxyethyl Metha Acrylate) as the Complexing Monomer. *Baghdad Sci. J.* **2018**, *15* (1), 63–72.

(16) Q8, I. C. H. International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Topic Q9: Quality Risk Management Geneva **2005**, 17. https://doi.org/10.4103/0976-500X.162004
(17) Guideline, I. C. H. H. T. Validation of Analytical Procedures: Text and Methodology. *Q2* **2005**, *1* (20), 5.
(18) Committee, I. C. H. S. Validation of Analytical Procedures. In: *International Conference on Harmonization of Technical Requirements for Registration of Pharmaceutical for Human Use*; **1994**, 27.
(19) Kumari, B.; Khansili, A. Analytical Method Development and Validation of UV-Visible Spectrophotometric Method for the Estimation of Vildagliptin in Gastric Medium. *Drug Res. (Stuttg).* **2020**, *70* (9), 417–423. https://doi.org/10.1055/a-1217-0296
(20) Sawant, D. K.; Ige, P. P. Development and Validation of Simple UV-Spectrophotometric Method for Estimation Diclofenac Sodium in Bulk and Tablet Dosage Form. *Pharm Analysis & Quality Assurance* **2016**, *2016* (4), 1–4.
(21) Naveed, S.; Qamar, F. UV Spectrophotometric Assay of Diclofenac Sodium Available Brands. *J. Innov. Pharm. Biol. Sci.* **2014**, *1* (3), 92–96.