Available online at **www.joac.info**

ISSN: 2278-1862

Journal of Applicable Chemistry

2019, 8 (1): 203-213

(International Peer Reviewed Journal)

A Specific and Stability Indicating Assay Method for Quantification of Diclofenac Potassium in Soft Gelatin Capsules By UPLC

S. P. Vittal[1], Sumathi V Rao[1] and K. Ramakrishna[2*]

1. Aurobindo Pharma Limited Research centre, Hyderabad-500072, Telangana, **INDIA**
2. Institute of Science, GITAM (Deemed to be University), Visakhapatnam-530045, Andhra Pradesh, **INDIA**

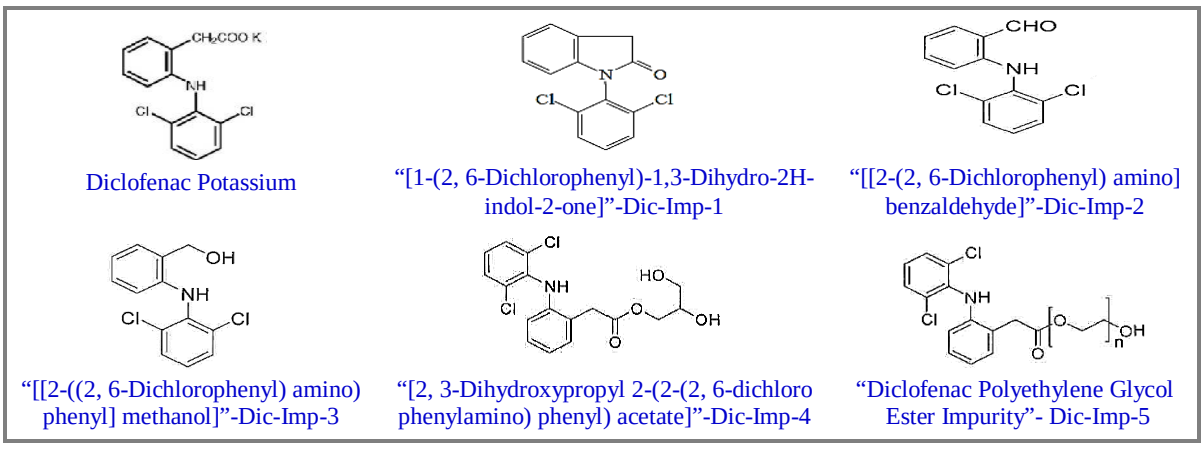
Email:karipeddirk@gmail.com

Accepted on 12th January, 2019

ABSTRACT

The principle idea of this research is to develop and validate a specific and shorter run time assay method for diclofenac potassium in soft gelatin dose formulation. A reverse phase assay methodology has been developed for quantification of diclofenac potassium in soft gelatin capsules in gradient elution mode, with short run time, specific from potential impurities and placebo. The assay methodology is optimized using Ultra performance liquid chromatographic (UPLC) technique using 0.1% phosphoric acid (OPA) pH 6.0 adjusted with (Triethyl)amine (Elution phase A) and Acetonitrile as (Elution phase B). A reverse phase C18 column Waters high strength silica (HSS) T3, 100 x 2.1 mm, 1.8µ is used with a flow of 0.4 mL min^{-1}. Gradient elution technique along with 280 nm as working wavelength, 1µL sample is injected by keeping column oven and sample temperatures at 50°C and 25°C respectively. In the developed method, intervention due to diluent, placebo and impurities were not found at elution of Diclofenac peak. Degradation studies indicate diclofenac is sensitive to acidic and thermal stress conditions. % RSD of method precision result for diclofenac is 0.17. Method is found to be linear. LOQ values shows that the method is having very good sensitivity. Method robustness was checked and found that the method is robust at variable conditions.

Graphical Abstract



Diclofenac Potassium | "[1-(2, 6-Dichlorophenyl)-1,3-Dihydro-2H-indol-2-one]"-Dic-Imp-1 | "[[2-(2, 6-Dichlorophenyl) amino] benzaldehyde]"-Dic-Imp-2

"[[2-((2, 6-Dichlorophenyl) amino) phenyl] methanol]"-Dic-Imp-3 | "[2, 3-Dihydroxypropyl 2-(2-(2, 6-dichloro phenylamino) phenyl) acetate]"-Dic-Imp-4 | "Diclofenac Polyethylene Glycol Ester Impurity"- Dic-Imp-5

Chemical structures of probable impurities of diclofenac potassium.

Keywords: Diclofenac potassium, Forced degradation, Stability, UPLC.

INTRODUCTION

Diclofenac potassium soft gelatin capsules belong to group of NSAID, available in 25mg strength for oral route administration. It is marketed under the brand name of Zipsor. It is used to treat for osteoarthritis pain associated with mild to moderate condition. Diclofenac potassium (Dic) is in crystalline structure available in powder form. It is present either in white or slightly yellowish in color. Solubility in water is less at 25°C and classified as sparingly soluble. The acid form of Dic has a pKa of 4. It is chemically defined as 2-[(2, 6-dichlorophenyl) amino] benzene acetic acid, has a molecular weight of 334.24 with empirical formula of $C_{14}H_{10}C_{l2}$ NKO_2. The structure of Diclofenac potassium is represented in figure 1.

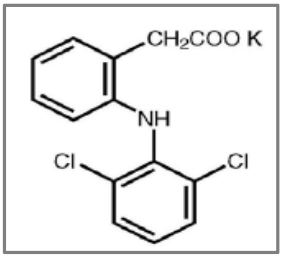


Figure 1. Structure of diclofenac potassium

On thorough literature research it was found that there are many UV spectrophotometric and HPLC methods are reported for quantification of Dic in different pharmaceutical products as well as in plasma [**1-11**]. Methods are reported to quantify different salt forms (sodium and Potassium salts) of Dic pharmaceutical dosage forms, both in single and multicomponent products. It is also reported that

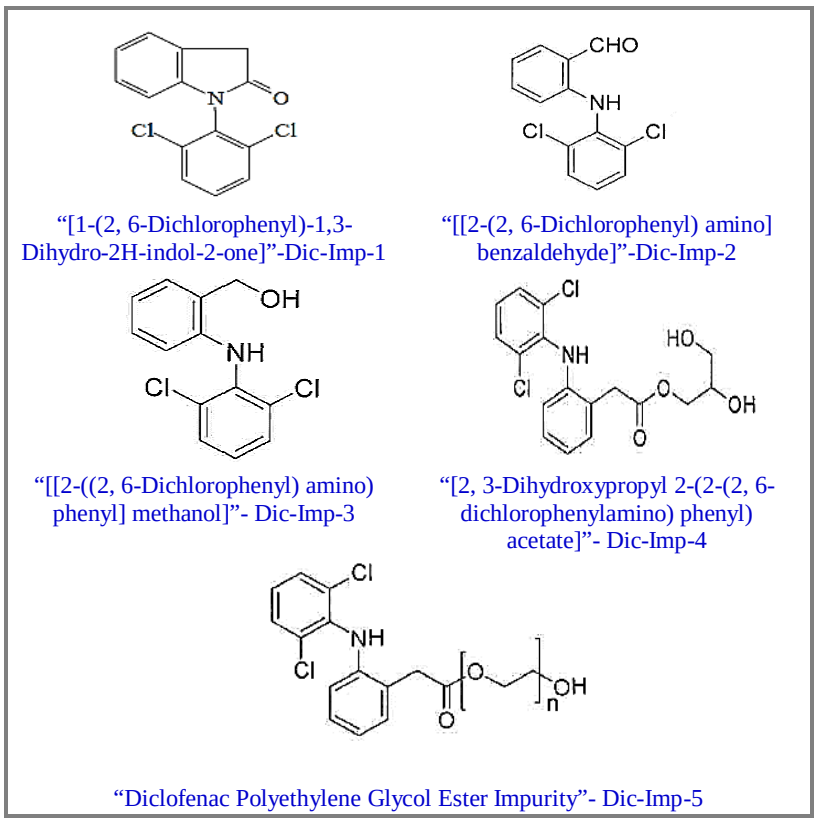


Figure 2. Chemical structures of probable impurities of Dic.

many UPLC methods for determination of the Dic (mostly sodium salt) in different single and multicomponent pharmaceutical dosage forms [**12-16**]. A LC-MS method is also reported for determination Dic in Human plasma [**17**]. The above reviews shows, that there is no precise, accurate and specific method with shorter run time for quantification of Dic in liquid filled capsules. The finalized method is very simple yet accurate to determine Dic in a complex formulation. Method is capable of separating all degradation impurities originating during stress and long- term stability conditions and are away from Dic peak in optimized chromatographic conditions. Hence method is more specific and stability indicating as well. The possible probable impurities of Dic originate through root of synthesis and its degradants and their chemical structures are represented in figure 2. Diclofenac related compound A(Dic Imp-1), Diclofenac related compound B(Dic Imp-2), Diclofenac related compound C(Dic Imp-3), Diclofenac glycerol ester impurity(Dic Imp-4) and Diclofenac polyethylene glycol ester impurity(Dic Imp-5)

MATERIALS AND METHODS

Reagents and Chemicals: Pure drug substance of Dic and individual impurities was sponsored by Aurobindo Pharma Limited. HPLC grade Phosphoric acid (OPA); GR grades chemicals of hydrogen peroxide, hydrochloric acid and sodium hydroxide; gradient grade solvents of Acetonitrile and Methanol were procured from Merck. Evoqua water purification system is used to get purified water for elution phase A and diluent preparations.

Instrumentation: Water acquity UPLC instrument with quaternary pump, auto injector, temperature controlled column compartment and Photo diode array detector used for evaluation of peak homogeneousness and pureness of analytes and for quantification purpose. Empower Pro software was used for acquiring the data. Waters High strength silica (HSS) T3, 100 x 2.1mm, 1.8µ was used for separation of main peak from placebo and impurity peaks.

Method development and optimization: There is no official monograph for Dic filled in capsules and no publications cited for assay methodology using UPLC technique. The aim of the work is to design short and stability indicating method to quantify Dic, specific from placebo peaks and potential impurities originating from drug product. Dic has pka about 4.26 and hence trials need to be taken either at pH below 2.5 or above 5.5 for having repeatability of main peak. Trial was initiated with 0.1% phosphoric acid and acetonitrile in the proportion of 50: 50 v/v with water acquity BEH C18 (2.1 x 50) mm, 1.8 µ column with flow of 0.2 mL min^{-1}, column temperature of 30°C.Dic eluted at about 2.9 min but Dic imp-1, 5 and 3 were coeluting on either side of main analyte and run time of 9 minutes required for complete run. In order to have separation of main analyte from the potential impurities gradient elution mode was adopted using the same column. Specificity could not be achieved. Further trials were taken with 10mM sodium dihydrogen phosphate buffer pH 3.0 and acetonitrile in gradient elution using same column and flow. Specific method could not be achieved by changing gradients, flow and column temperature.

Further trials were continued at elevated pH i.e above pH 5.5 by using 0.1% phosphoric buffer with pH adjusted to 6.8 with (Triethyl) amine using BEH C18 (2.1 x 50) mm, 1.8 µ using flow rate of 0.4 mL min^{-1}. Dic eluted at about 3.75 min and specific from all the potential impurities. The USP tailing of Dic peak is found above 1.7. In order to attain symmetrical peak shape and impurity specific method, trials performed using on Waters High strength silica (HSS) T3, 100 x 2.1mm, 1.8µ using gradient elution. Finally, sharp and symmetric peak was observed. In the same gradient programme trial was taken with reduced pH of buffer i.e. from pH 6.8 to 6.0. Dic eluted at about 2 min and all impurities started eluting after 3.2 min and run time was completed within 6 min.

The optimized method is found specific from diluent, placebo and potential impurities originating from drug product. The finalized method is achieved with 0.1% phosphoric acid pH adjusted to 6.0 with (Triethyl)amine, acetonitrile in gradient elution along with Waters High strength silica (HSS) T3, 100 x 2.1 mm, 1.8µ column. The flow is 0.4 mL min^{-1}, column temperature 50°C. Dic peak shows

maxima at 280 nm, hence the same wavelength is opted. Main analyte was satisfactorily separated from the peaks of probable impurities. Hence, the same mobile phase in gradient elution along with Waters High strength silica (HSS) T3, 100 x 2.1mm, 1.8µ column was finalized.

Dic is soluble in aqueous medium at pH about 6.8. For complete recovery of the drug from the soft gelatin capsules, 20 mM phosphate buffer with pH 6.8 and methanol in the proportion of 50:50 v/v was used as diluent-1. In order to attain a better peak shape for Dic, a second dilution was proposed using water and methanol in the proportion 80:20 v/v. From spectral data of the drugs, it was found that, Dic shows wavelength maximum at 280 nm and hence the same wavelength was opted for quantification purpose (Figure 3)**.** The samples were prepared at the concentration of 50 mg mL^{-1} and for this concentration injection volume of 1 µL was sufficient to attain satisfactory areas.

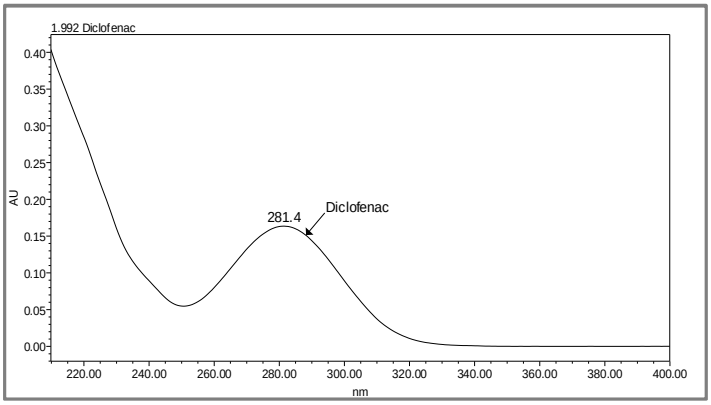


Figure 3. Spectra of diclofenac potassium.

Optimized chromatographic conditions were validated as per compendia requirement such as ICH [**18-22**].

Finalized chromatographic conditions: Elution phase-A is 0.1% orthophosphoric acid (1 mL of orthophosphoric acid added to 1 L of water) pH is adjusted to 6.0 with (Triethyl) amine. Acetonitrile was used for elution phase-B. The column oven was controlled at temperature of 50°C. Dic was specific from placebo and potential impurities in gradient elution mode at constant flow rate of 0.4 mL min^{-1}. Column loaded with 1 µL injection volume and the analyte was detected at 280 nm wavelength. The finalized chromatographic conditions are given in table 1 and observed retention time for Dic is about 2 min. System suitability results presented in table 2.

Table 1. Finalized method parameters

Column	Waters High strength silica (HSS) T3, 100 x 2.1mm, 1.8µ		
Flow	0.4 $mLmin^{-1}$		
Column oven temperature	50°C		
Injection volume	1 µL		
Wavelength	280 nm (PDA Detector)		
Elution phase-A	0.1% Phosphoric acid in water, pH adjusted with (Triethyl)amine to 6.0.		
Elution phase-B	Acetonitrile		
Gradient programme	Time Interval (min)	% Elution phase-A	% Elution phase-B
	0.0	65	35
	1.0	55	45
	2.0	40	60
	2.5	30	70
	3.0	20	80
	4.0	20	80
	4.2	65	35
	6.0	65	35

Table 2. System suitability results

Analyte name	Retention Time (min)	Theoretical plates	Tailing factor
Dic	2.00	42298	1.35

Preparation of solutions

Standard solution: 0.5mgmL^{-1} standard stock solution is prepared using diluent-1. This is further diluted to obtain a concentration of 50 µg mL^{-1} using diluent-2.

Sample solution: 10 intact capsules were transferred into a 500 mL volumetric flask and added 180 mL of 20 mM phosphate buffer and allowed to dissolve by sonication. Further 120 mL of methanol is added and sonicated the solution at least 30 min. The solution is intermittently mixed to get homogeneity of the solution. The resultant solution is equilibrated to room temperature and made up to the volume using diluent-1. Further dilution was performed by taking 5 mL of above sample stock solution to 50 mL with diluent-2.

Impurity solutions: Individual impurity was prepared initially by dissolving in methanol, followed by further dilution with diluent-2 to a concentration of 0.5 µg mL^{-1}. Impurity solution of individual impurity was injected to UPLC system to check the specificity.

System suitability parameters: Standard solution was injected for five times into UPLC system for evaluation of system suitability parameters such as % RSD, Column efficiency (Plate count) and Tailing factor.

Specificity (Interference from Placebo and degradants): Placebo solution and individual impurity solutions were prepared and injected into UPLC to perform the specificity experiment. Forced degradation study was conducted on the capsules and the solutions were analyzed to study the degradation behavior of Dic.

Acid stress: To this study, 20 mL of 5 M hydrochloric acid was added to the sample preparation. The solution was placed on water bath maintained at 85°C for about 10 minutes. The stressed sample was equilibrated to room temperature and neutralized with 20 mL of 5M Sodium hydroxide solution. The sample solution was made up to volume with diluent-1 and further diluted with diluent-2.

Alkaline stress: To this study, 20 mL of 5 M sodium hydroxide solution was added to the sample preparation. The solution was placed on water bath maintained at 85°C for about 180 min. The stressed sample was equilibrated to room temperature and neutralized with 20 mL of 5M hydrochloric acid. The sample solution was made up to volume with diluent-1 and further diluted with diluent-2.

Oxidative stress: To this study, 20 mL of 30% hydrogen peroxide solution was mixed with the sample preparation. The solution was exposed to 85°C for about 180 min on a water bath. Later the solution was allowed to equilibrate to room temperature. The sample solution was made up to volume with diluent-1 and further diluted with diluent-2.

Thermal stress: Capsules were exposed to 120 h at hot air oven at a temperature of about 85°C. Sample solution with the exposed capsules were prepared as per testing procedure

Photolytic and humidity stress: Capsules were collected in two glass plates. One sample loaded in Photo chamber and exposed to white fluorescent light of 1.2 million lux h and UV light of 200-Watt Hr. m^2. Another sample was kept in Humidity chamber at 95%RH/25°C for 5 days. The above degraded stock solutions were prepared as per testing procedure.

Method precision: Method precision was performed on intact capsules in six individual preparations. The %RSD was calculated. The Ruggedness of the method was checked by repeating the Precision

experiment on another day by second analyst with different column and different UPLC instrument.

Recovery: Accuracy study was performed by addition of known amount of pure analyte to fixed quantity of placebo (which is equal to the amount present in the Capsule) at 50%, 100% and 150% concentration levels. These solutions were injected into UPLC system and recovery values were calculated from "amount found" relative to "amount added".

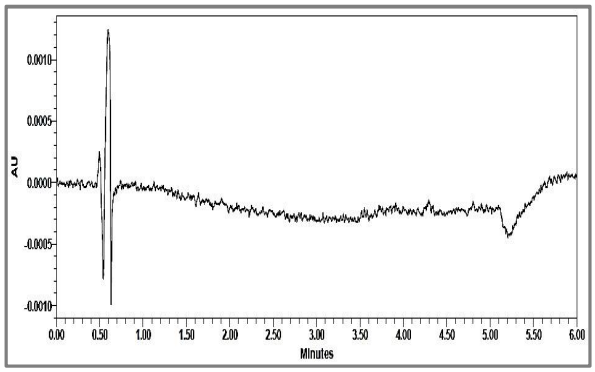
Linearity from response of the detector: Linearity curves were generated from 25% to 150% of the sample concentration. Correlation coefficient, slope and Y-intercept from the Linearity plots were assessed.

Stability of solution: Stability of the solution was assessed by injecting periodically till 40h at 25°C for standard and sample, which were prepared as per testing procedure.

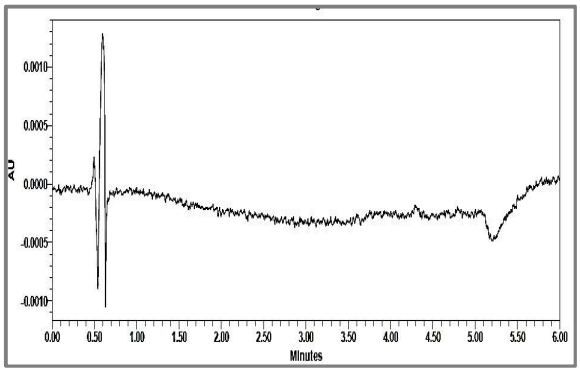
Robustness of the method: The chromatographic parameters such as flow rate, column temperature, organic composition and wavelength were changed as mentioned below during Robustness study. The elution phase flow is maintained ±12.5% of actual flow rate of 0.4 mL min^{-1} (i.e., 0.35 and 0.45 mL min^{-1}); Column oven temperature (50°C) adjusted ± 5°C i.e., 45°C and 55°C; the composition of elution phase B ± 2 % (absolute) in gradient program; change in wavelength (280 nm) of ± 5 nm i.e., 275 nm and 285 nm; change in pH (6.0) of ± 0.5 units i.e. 5.5 and 6.5. The system suitability parameters were evaluated in each of the varied condition.

RESULTS AND DISCUSSION

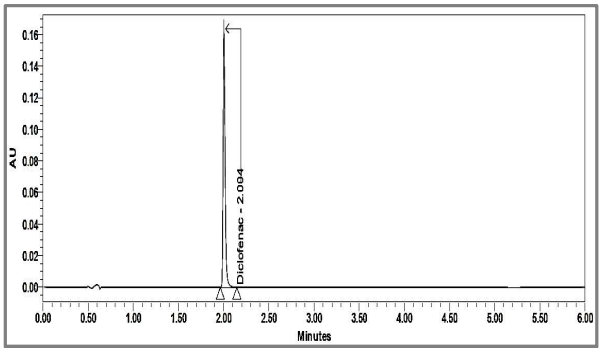
Specificity and stress data: From the specificity experiment, no interference was observed at the retention times of main peak from diluent, placebo and probable impurities. Impurities related to Dic as specified in figure 4k are well separated from the main analyte peak and also degradants generated during forced degradation studies. Stress study was conducted on Capsules formulation by acid



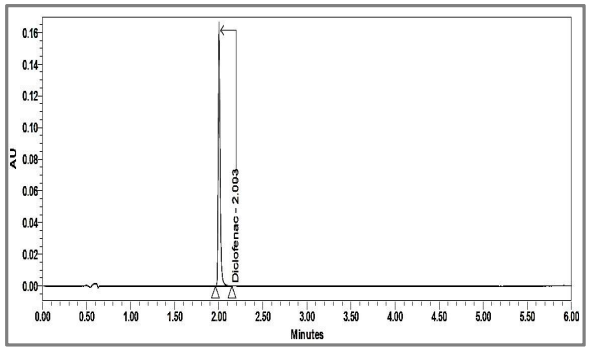
4a. Diluent



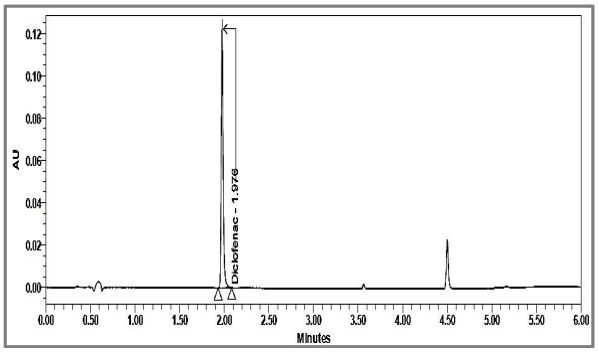
4b. Placebo



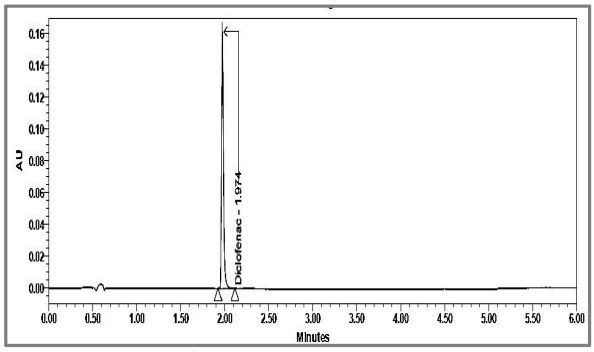
4c. Standard



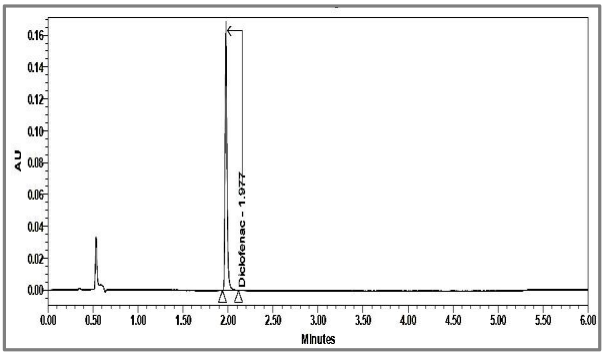
4d. Sample



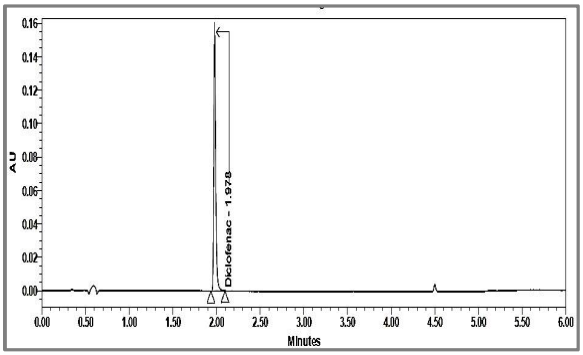
4e. Acid Stressed Sample



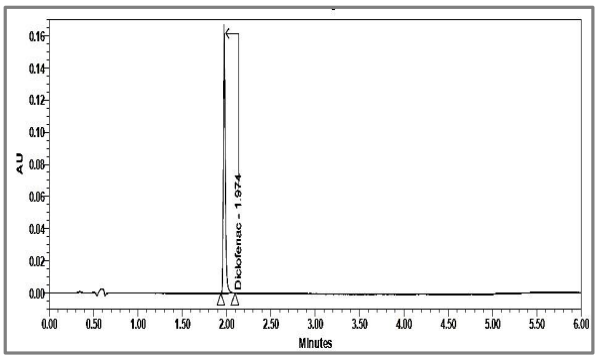
4f. Base Stressed Sample



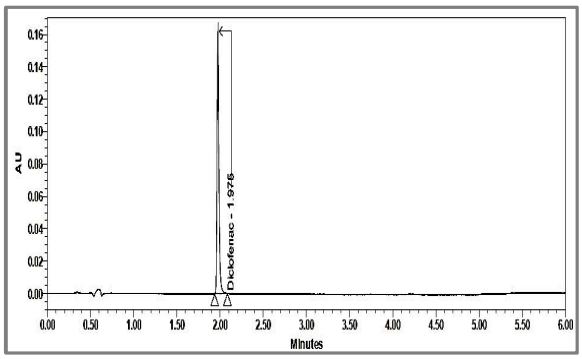
4g. Peroxide Stressed Sample



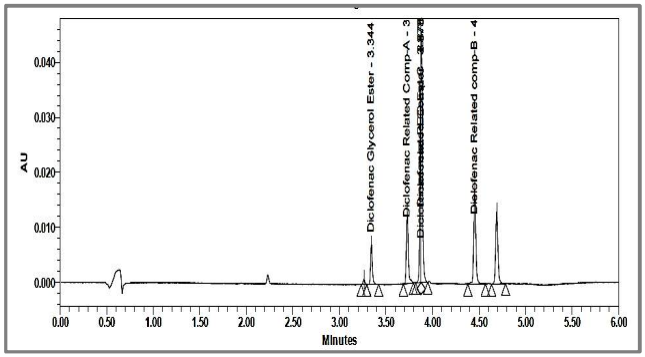
4h. Thermal Stressed Sample



4i. Photolytic Stressed Sample



4j. Humidity Stressed Sample



4k. Probable Impurities of Dic

Figure 4. Chromatograms of diluent, Placebo standard Sample and Stressed samples.

hydrolysis, base hydrolysis, and oxidation by Hydrogen peroxide, exposure to Heat, Humidity and Light. From the above stress studies it indicates that Dic is sensitive to acid and thermal conditions. Chromatograms of standard, sample and stress samples were depicted in figure 4c-4j. The degraded samples were evaluated for peak purity of Dic and found to be pure and there are no co-eluting peaks.

The % of degradation in each degradation has been mentioned in table 3.

Table 3. Forced degradation results

Stress condition	% Degradation	Purity Angle	Purity Threshold
Acid (5M HCl, 85°C, 10 min)	15.1	0.109	1.050
Base (5M NaOH, 85°C, 180 min)	0.5	0.110	1.040
Peroxide (30% H_2O_2, 85°C, 180 min)	--	0.111	1.062
Thermal (85°C, 120 h)	4.3	0.106	1.045
Humidity (90% RH, 25°C, 120 h)	0.2	0.107	1.042
Photolytic (white fluorescent 1.2 Million lux hours UV 200 Watt h/m^2)	--	0.110	1.045

Summary of validation data: %RSD of method precision results for Dic is 0.17 respectively. %RSD during intermediate precision results is found to be 0.14. Results were given in table 4. Accuracy results were found between 98.0 to 102.0. Results were summarized in table 5. Method is found to be linear from 25% to 150% of test concentration of test solution with correlation coefficient values of greater than 0.999. Slope and intercept values were also reported from Linearity experiment. Linearity results were depicted in table 6 and linearity plots were illustrated in figure 5. LOD and LOQ values were found to be 0.25 and 0.50 µg mL^{-1} for Dic which shows that the method is having very good sensitivity and data is given in table 7. Method robustness was checked by making changes to flow

Table 4. Method precision and ruggedness (Intermediate precision) results

Sample	Precision	Ruggedness
	% Assay	% Assay
ID#	**Dic**	**Dic**
1.	99.1	100.0
2.	99.0	99.7
3.	99.1	99.8
4.	99.1	99.7
5.	98.9	99.6
6.	99.4	99.8
Average	99.1	99.8
SD	0.17	0.14
%RSD	0.17	0.14

Table 5. Recovery (Accuracy) results

Drug	Amount added (mg)	Amount found (mg)	% Recovery
Dic	125.00	123.05	98.44
	250.00	248.17	99.27
	375.00	375.17	100.04

Table 6. Results for linearity

Concentration (µg mL^{-1})	Response
12.605	66987
25.210	134484
50.420	268669
60.504	323641
75.630	406963
Correlation	1.000
Slope	5382.99
%y-Intercept	-0.52

rate, column oven temperature, buffer pH, mobile phase composition and working wavelength. Robustness data was given in table 8. Stability of solutions were evaluated and found both solutions were stable for 40 h at 25°C. From the results of method validation performed as per ICH guideline, showed satisfactory results in all parameters.

Table 7. LOD and LOQ precision results

Sample	LOD	LOQ
	Area	Area
ID#	Dic	Dic
1.	1289	2534
2.	1220	2679
3.	1249	2582
4.	1287	2493
5.	1293	2566
6.	1295	2560
Average	1272	2569
SD	30.71	62.23
%RSD	2.41	2.42

Table 8. Robustness study for diclofenac potassium

Method parameter	Retention time (minutes)	Theoretical plates	Tailing factor
As per method	1.96	38342	1.36
Wavelength (285 nm)	1.96	38335	1.36
Wavelength (275 nm)	1.95	38018	1.35
Flow rate (0.45 mL.min^{-1})	1.77	35083	1.32
Flow rate (0.35mL.min^{-1})	2.19	40167	1.31
Organic composition (+2%)	1.75	25924	1.27
Organic composition (-2%)	2.17	44256	1.28
Temperature (55°C)	1.95	35145	1.29
Temperature (45°C)	1.99	33662	1.26
pH (6.5)	1.86	49503	1.13
pH (5.5)	2.28	31933	1.28

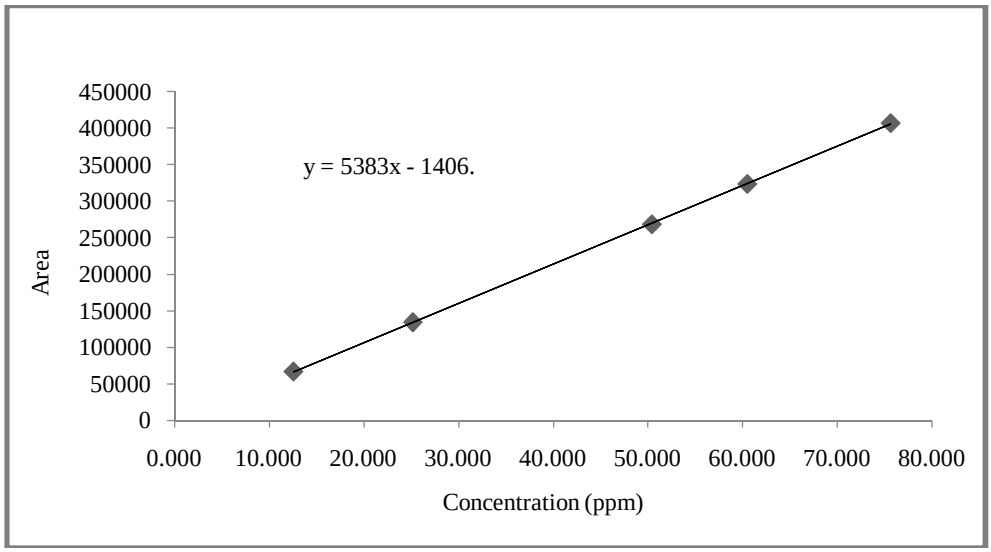


Figure 5. Linearity plot of diclofenac potassium

APPLICATION

The projected method is suitable for routine and stability analysis purpose due to its high sensitivity and shorter run time, it can be very useful in assessing the content of Dic in soft gelatin formulation.

CONCLUSION

The developed and validated UPLC method is appropriate for quantification purpose and stability demonstrating. The samples at different shelf life conditions can be tested using this method without any interference. Also, short run time of the assay method enables analysis of a greater number of samples at faster rate. Due to its high sensitivity and shorter run time, this method can be very useful in assessing the content of Dic in soft gelatin formulation.

ACKNOWLEDGEMENTS

The author is highly obliged to Aurobindo Pharma Limited Research centre for extending support for completion of this work.

REFERENCES

[1]. Devi M. Gayathri, Latha P. V. Madhavi, Sony N. Pravallika, G. Rohini, I. Indu, Krishna B. Vamsi, A. C. S. Rupika, Devi P. Uma, Analytical method development and validation for the estimation of diclofenac sodium in bulk and tablet dosage form by RP-HPLC, *European Journal of Biomedical and Pharmaceutical Sciences*, **2018**, 5(4), 1-5.

[2]. Battu, Sowjanya,Yalavarthi, Prasanna Raju, Subba, G. V. Reddy, An isocratic, validated RP-HPLC technique for simultaneous estimation of diclofenac and nifedipine in pharmaceutical formulation, *Journal of Global Trends in Pharmaceutical Sciences*, **2017**, 8(3), 4160-4165.

[3]. Arisoy, Guelsuem Guel, Dural, Emrah, Mergen, Goerkem, Arisoy, Mustafa, Guevendik, Guelin, Soeylemezoglu, Tuelin, Development and validation of HPLC-UV method for the determination of diclofenac in human plasma with application to a pharmacokinetic study, *Turkish Journal of Pharmaceutical Sciences*, **2016**, 13(3), 292-299.

[4]. V. A. Khanin, O. M. Kotenko, O. O. Moiseev, O. V. Dorovskyy, Development of the original method for determination of diclofenac sodium in different drug dosage forms, *Visnik Farmatsii*, 2016, (4), 46-49.

[5]. L. V. Brun, V. I. Makolinets, S. N. Gubar, Pharmacokinetic study of diclophenac sodium in rat plasma by high-performance liquid chromatography, *Ukrainian Biopharmaceutical Journa*l, **2016**, (4), 62-70.

[6]. P. Palanisamy, B. Jayakar, M. Kumar, R. Margret Chandra B. S. Venkateshwralu, A. Pasupathi, M.V. Kumudhavalli, Development of a RP-HPLC Method for the estimation of Diclofenac sodium, Vitamin B1, Vitamin B_6 and Vitamin B_{12} Insoft Gelatin Capsule Dosage, *International Journal of Pharmacy Teaching and Practices* , **2014**, 5(3), 1002-1013.

[7]. S. Derraji, El M' M. Rabet, A. Dahchour, Y.Cherrah, A. Bouklouze, Validation of an UV-Visible method for the assay of diclofenac sodium: Its use in decontamination rate of this active ingredient by natural adsorbents, *Journal of Materials and Environmental Science*, **2015**, 6(3), 818-825

[8]. Alexandre Machado Rubim1, Jaqueline Bandeira Rubenick, LucianeVarine Laporta, Clarice Madalena Bueno Rolim, A simple method for the quantification of diclofenac potassium in oral suspension by high-performance liquid chromatography with UV-detection, *Brazilian Journal of Pharmaceutical Sciences*, **2013**, 49, 589-597.

[9]. R. Sanjay Kumar, C. Karthikeyan, N. S. H. N. Moorthy, P. Trivedi, New spectrophotometric methods applied to the simultaneous determination of diclofenac potassium and tizanidine, *Indian J Pharm Sci*, **2011**, 73 (3), 300-302

[10]. SafilaNaveed, Hina Rehman, Fatima Qamar, Syeda Zainab. Development of a spectrophotometric method for the assay of Diclofenac potassium, Journal *of Innovations in Pharmaceuticals and Biological Sciences*, **2015**, 2(1), 7-11.
[11]. Sagar Suman Panda, DebasisPatanaik, Bera V. V. Ravi Kumar, New Stability-Indicating RP-HPLC Method for Determination of Diclofenac Potassium and Metaxalone from their Combined Dosage Form, *Sci Pharm.*, **2012**, 80(1), 127–137.
[12]. Elzayat, M. Ehab, Ibrahim, F. Mohamed, Abdel-Rahman, A. Ali Ahmed, M. Sayed, Alanazi, K. Fars, Habib, A. A. Walid, Validated stability-indicating UPLC method for determination of diclofenac sodium in its pure form and matrix formulations, *Arabian Journal of Chemistry*, **2017**, 10(2), S3245-S3254.
[13]. V. A. Khanin, O. M. Kotenko, O. O. Moiseev, O. V. Dorovskyy, Visnik Farmatsii Development of the original method for determination of diclofenac sodium in different drug dosage forms, 2016, (4), 46-49.
[14]. M. Azougagh, M. Elkarbane, K. Bakhous, S. Issmaili, A. Skalli, S. IbenMoussad, B. A. Benaji, novel stability-indicating UPLC method development and validation for the determination of seven impurities in various diclofenac pharmaceutical dosage forms, *Annales Pharmaceutiques Francaises*, **2016**, 74(5), 358-369.
[15]. Gu, Ji-hong, Dong, Ji, Miao, Li-yan Determination of diclofenac's concentration in plasma by UPLC-MS/MS, *Shenyang Yaoke Daxue Xuebao*, **2014**, 31(12), 979-983
[16]. Shuyao, Sun, Jinhong, Peng, Lei, Wang, Xiaoya, Determination of related substances in diclofenac sodium by UPLC, *Chin from Guangdong Huagong*, **2014**, 41(4), 87-88.
[17]. T. Bhanu Praksah, K. Vijaya Sri, S. Rama Krishna Determination of Diclofenac Potassium in Human Plasma by LC-MS, Asian Journal of Chemistry, **2009**, 21(6), 4183-4189.
[18]. Validation of Compendial Methods 1225, *The United States Pharmacopeia*, **2012.**
[19]. Photo stability Testing of New Drug Substances and Products, ICH, Q1B, 2005.
[20]. Analytical Procedures and Methods Validation, *US FDA Guidance*, **2000**.
[21]. Validation of Analytical Procedures: Text and Methodology, *ICH*, Q2 (R1), **2005**.
[22]. Stability Testing of New Drug Substances and Products, *ICH*, Q1A (R2), **2005**.