DEVELOPMENT AND VALIDATION OF STABILITY INDICATING METHOD FOR THE ESTIMATION OF ACECLOFENAC IN MARKETED FORMULATION

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Article History: **Received:** 26.04.2023 **Revised:** 21.06.2023 **Accepted:** 17.07.2023

Abstract:

This is the study where we developed and validated an reversed phase high performance liquid Chromatography method with Ultraviolet detection for the Estimation of Aceclofenac drug in marketed formulation. The method was developed on kromasil C18 analytical column (150 X 150mm,4.6 µm) with mobile phase consisting of mobile phase A, buffer: methanol in ratio 90:10 and mobile phase B, ACN: methanol 90:10% v/v. The flow rate was kept at 1 ml/min and the column was maintained at 40°C. The detection carried out at wave length of 275 nm. Calibration plot was linear in concentration range of 50–150ppm levels with the correlation coefficient (r^2) of 0.999.The method was validated with regards to system precision, method precision, linearity, accuracy, limit of detection, quantification and robustness and can be used for estimation of Aceclofenac drug in bulk and tablet dosage form. The forced degradation studies are also performed.

Keywords: Aceclofenac, High Performance Liquid Chromatography, Gradient, Estimation, correlation coefficient, forced degradation, ICH guideline.

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DOI: 10.31838/ecb/2023.12.s3.712

1. INTRODUCTION

Aceclofenac is a type of drug which comes under Non-steroidal anti-inflammatory drug (NSAID) similar to Diclofenac, It is used for rheumatoid arthritis, inflammation and relief pain, it was patented for ankylosing osteoarthritis in the year 1983 and approved for medical use in 1992[1].The drug works by inhibiting the action of cyclooxygenase (COX) that is involved in the production of prostaglandins (PG) which is accountable for pain, swelling, inflammation and fever[2].Literature survey reveals that ,UV spectrophotometric[3-4] ,HPLC[5-7] , Thin layer chromatography[8] , Spectrofluorimetric[9] , LC-MS[10]have been reported for the determination of Aceclofenac. Therefore few HPLC methods were available for the estimation of Aceclofenac alone either in bulk or in dosage forms. Therefore, an attempt has been made to develop an accurate, simple, precise, cost effective, reproducible reverse phase HPLC method for estimation of Aceclofenac in dosage form and validate it, in accordance with ICH guidelines[11].Chemical formula for Aceclofenac is $C_{16}H_{13}Cl_2NO_4$, molecular mass is 354.18 g·mol^{-1}and IUPAC name is [(2-{2, 6-dichlorophenyl) amino} phenylacetooxyacetic acid][12] .

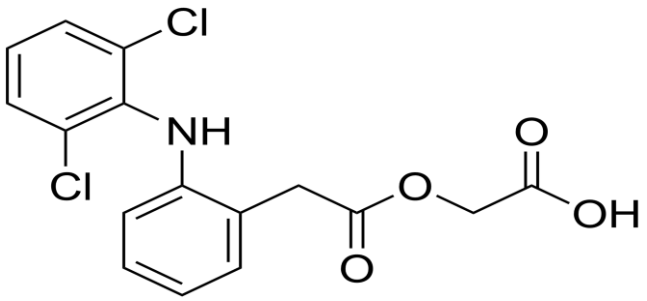

Fig 1: Structure of Aceclofenac

2. MATERIALS AND METHODS

Material:
Aceclofenac API and tablets were received as a gift sample from Umedica laboratories Pvt. Ltd., R&D center, Turbhe India. All the chemicals were of analytical grade from Merck, Mumbai, India.

Instrumentation:
The HPLC system (Shimadzu LC Solution 2010) consisted of a pump along with auto-injector sampler. The detector consisted of UV /Vis at 276 nm and forced degradation was perform on PDA detector.LC separation were performed on kromasil C18 analytical column (150 X 150mm,4.6 µm).The software used was lab-solution software.

Selection of wave length:
Standard Solution of Aceclofenac (50ppm) were prepared and scanned by UV spectrophotometer, in the range of 200-400nm and UV spectra of Aceclofenac was obtained as shown in Fig.2, 275 nm wavelength was selected as detection wavelength for separation of Aceclofenac.

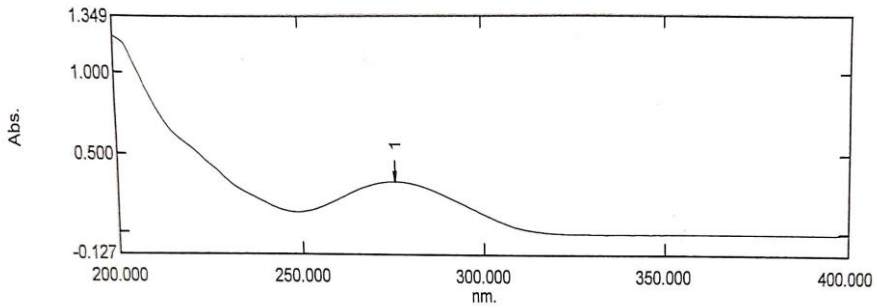

Fig 2: Spectrum of Aceclofenac API

Chromatographic conditions:
Aceclofenac drug was separated with all the impurities on kromasil C18 analytical column (150 X 150mm,4.6 µm) particle size analytical column. The analyst was eluted with the mobile phase consisting of phosphate buffer(6.5 pH) , mobile phase A Buffer: Methanol in ratio 90:10 and mobile phase B Acetonitrile: methanol 90:10% v/v with a gradient program set as time/% of mobile phase B: 0/25,5/42,9/75,16/75,17/25,22/25 . Auto sampler and column temperatures were set at 15°C. The injection volume was used 10 µl and the detector was set at wavelength of 275 nm. The run time was 22 minutes.

Preparation of buffer solution:
Accurately weigh and transfer 0.69 gm of Sodium Di-hydrogen phosphate monohydrate and 0.71 g of Disodium hydrogen phosphate anhydrous in 1000 mL of purified water, sonicate to dissolve and mix well. Adjust pH to 6.50± 0.05 with diluted OPA solution and mix well the solution.Filter buffer through 0.45µm Nylon membrane filter.

Preparation of standard solution:
50mg of Aceclofenac pure drug was weighed and transferred into 50ml volumetric flask add 30ml of diluent and sonicate to dissolve completely for about 10min.Make up the volume up-to the mark with diluent and mix well. Pipette out 5 ml of standard stock solution and transfer into a 25 ml volumetric flask and dilute up to the mark with diluent and mix well. From this stock solution various aliquots are prepared and injected. (concentration of Aceclofenac about 200 µg/ml**).**

Preparation of sample solution of Aceclofenac:
Select 10 tablets randomly and determine average weight. Transfer 10 intact tablets into 200 ml of dry and clean volumetric flask. Add 140ml of diluent and Sonicatefor 30min with intermittent shaking. Allow to cool the sample and make up the volume up to the mark with diluent. Centrifuge this solution at 4000 RPM for 10 minutes. Filter the solution through 0.45 um filter. Pipette out 4ml of filtrate and transfer into a 100ml volumetric flask and make up the volume with diluent and mix well and fill the HPLC vial.

Blank/Diluent: ACN:Water 50:50

3. Result And Discussion

Method validation:
The HPLC developed method was validated for, linearity, specificity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and system suitable parameters with the ICH guidelines Q2 (R1).

System suitability:
Firstly the HPLC system was optimized as per the chromatographic conditions. 10 µL of standard and sample solution of aceclofenac drug were injected in six injections into the HPLC system. To discover the system suitability for the advanced method, the parameters such as retention time, theoretical plates, resolution and tailing factors were calculated. The obtained results are in line with ICH guidelines and illustrate the good performance efficiency. The system suitability results are listed in Table 1.

Table No. 1: Results of System Suitability

Parameter	Result	Acceptance Limit
Retention time (Rt)*	7.55	--
Number of theoretical plates (N)	21438	More than 2000
Tailing factor (T)*	1.70	Less than 2

* Number of injections: 6 replicates

Specificity:
Specificity was authenticated by comparing the retention time of the standards solution with the retention time of sample solution.

The specificity of the method was established by injecting the blank solution, standard and sample solution of the Aceclofenac drug. Specificity demonstrates no interference of impurity or any endogenous peak in the

retention time of Aceclofenac drug peak. The resulted chromatograms for blank and sample solutions were shown in the Figure No.3, and 4 respectively.

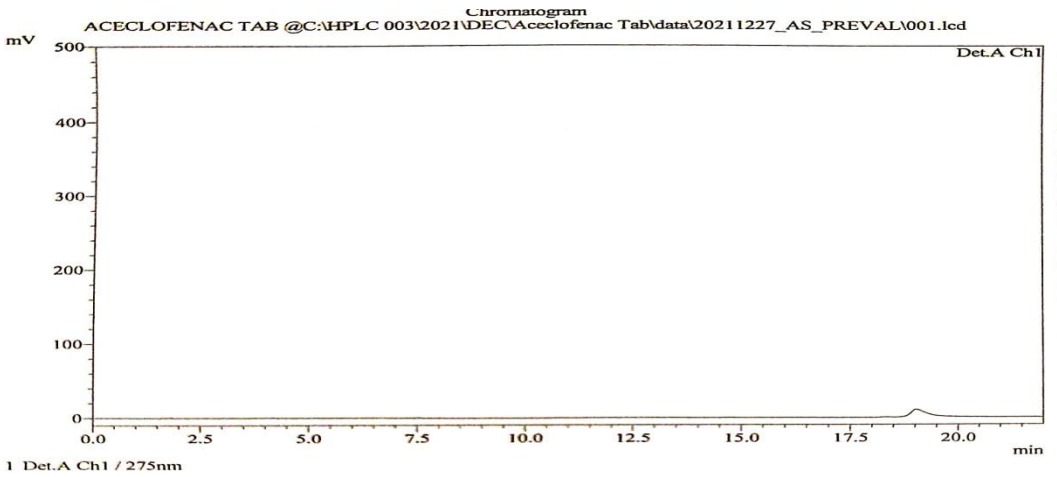

Fig. No.3 Chromatogram showing blank injection

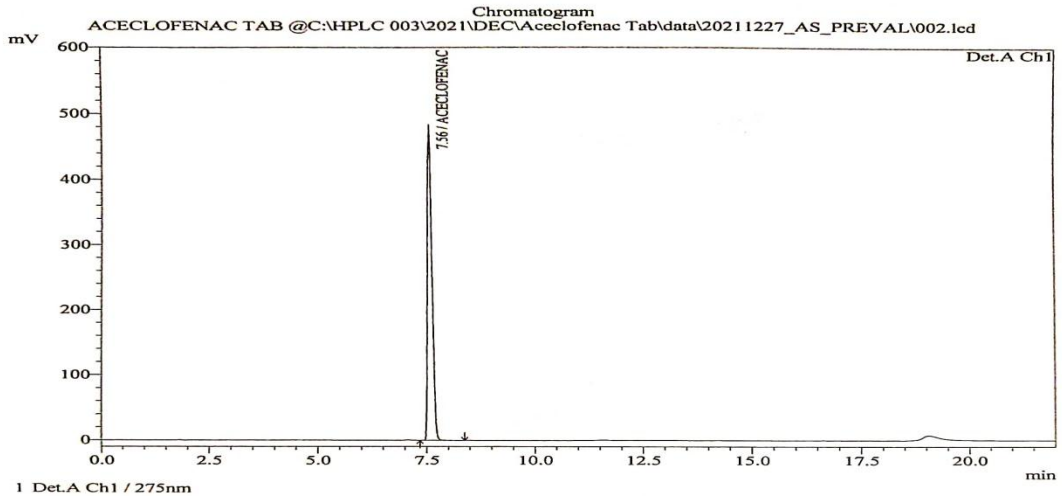

Fig. No.4 Chromatogram showing sample injection of Aceclofenac drug

Linearity:

The linearity curve of Aceclofenac was found to linear in the concentration range of 20-60 ppm. The calibration curve was plotted against concentration and area response as shown in the figure 5.

Make ready 20, 32, 40, 48 and 60 µg/ml concentration levels of calibration standard solutions of Aceclofenac and injected into the chromatographic system. A linear regression was used to plot the calibration graph of peak area (on Y- axis) versus concentration (on X-axis) of Aceclofenac. Each peak area was used to calculate the correlation coefficient (r^2). The results for linearity range and linearity were presented in the Table No.2 and fig no 5.

Table No.2: Results of Linearity Range

Concentration (µg/ml)	Area
20	1826988
32	2949023
40	3657652
48	4430234

60	5517141
Statistical analysis	
Slope	91522
y-intercept	22513
Correlation coefficient	0.9999

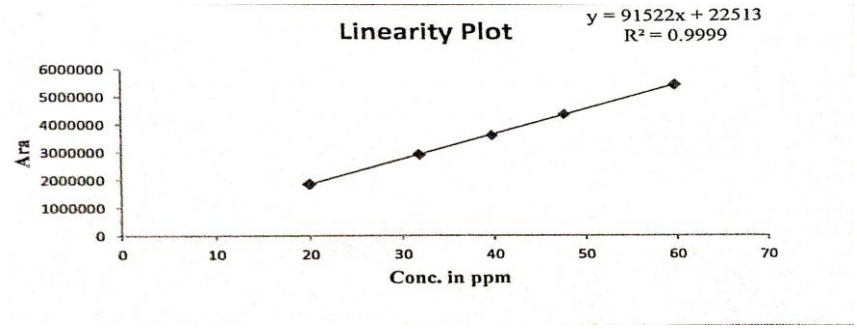

Fig 5: linearity curve of Aceclofenac

Accuracy (recovery):

Accuracy of the assay method can be checked by comparing three different test concentration. Known amount of standard solution of Aceclofenac at 50% 100% and 150 % were added to pre quantified, sample solution of Aceclofenac and injected into the chromatographic system. Each standard solution was prepared in triplicate and analyzed. The peak area of each level was used to calculate the percentage of recovery. According to ICH guidelines percentage of recovery should be in the range of 98 – 102 %. The obtained results were summarized in the Table No.3.

Table no 3: Result of accuracy

Brand Name	Label claim (mg)	Level	Amount Added (µg/ml)	Peak area	Amount Recovered (µg/ml)	Recovery	% Mean Recovery	% Mean RSD
Aceclofena-c tablet	100	50	2.57	1801654	2.55	99.2	99.2	100.03
			2.57	1814844	2.56	99.6		
			2.57	1817327	2.54	98.8		
		100	5.14	3650949	5.22	101.5	100.7	
			5.14	3663316	5.11	99.4		
			5.14	3643837	5.21	101.3		
		150	7.71	5450813	7.72	100.1	100.2	
			7.71	5425679	7.76	100.6		
			7.71	5393276	7.71	100.0		

Precision:

Method precision for Aceclofenac drug solution was checked by repeatability. We prepared six injection samples of same concentration of 200µg/ml of Aceclofenac drug solution and injected into the chromatographic system. The peak area of each injection was used to calculate the % RSD. For evaluating the intermediate precision we analyzed six injections of 200 µg/mL concentration of Aceclofenac drug on different days by different columns of same dimensions by different analysts. Each injection area was used to calculate the % RSD. From the data given in Table No.4 the developed method was found to be precise.

Table No. 4: Results of intraday and interday precision

Sr.No.	Intraday precision Area	Interday precision Area
1	3498795	3598995
2	3476826	3577726
3	3423676	3550759
4	3453178	3570133
5	3454189	3585749
6	3496421	3648582
Mean	3467181	3588657
Std Dev	29000	33482
%RSD	0.83	0.93

Ruggedness and Robustness:

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst. Robustness is a measure of capacity of a method to remain unaffected by small but deliberate variations in the method conditions, and is indications of the reliability of the method. The content of the drugs were not adversely affected by these changes as evident from the low values of % relative standard deviation (less than 2%). Thus results were shown in table no 5.

Table No.5: Results for Robustness

Sr. No.	Control	Flow rate (±10%)		Column temperature(±5°C)		Wavelength (±2nm)	
	1.0mL/min	**0.9mL/min**	**1.1mL/min**	**35**°C	**45**°C	**273nm**	**277nm**
1	3504436	3877497	3197199	3567383	3579534	3461164	3595512
2	3505263	3881834	3201148	3555878	3578917	3467600	3591695
3	3506426	3879207	3224838	3557306	3589654	3445213	3594532
Mean	3504285.8	3880893.4	321213.60	3561866.20	3587062	3457992	3593913
SD	2188.30	2900.10	13140.35	5487.68	7460	11525.58	1982.358
%RSD	0.06	0.07	0.41	0.15	0.21	0.33	0.05

Assay of Marketed formulation

The sample solution for assay was prepared according to above preparation of sample solution of Aceclofenac.

Table No.6: Results for Assay

Tablet	Drug	% Assay
Zerodol tablet	Aceclofenac	100.6%

Forced degradation:

Table No.7:Forced degradation study data

Sr no:	Type of Degradation	Degradation condition	% Degradation
1.	Acid treated	1N HCL(5mL) 24 hr	10%
2.	Base treated	0.5 N NaoH (1mL) 30 min	3%
3.	oxidative treated	30%H_2O_2(5mL) 1hr	6%
4.	Thermal treated	100°C for 24 hr	7%

Separation of impurities:

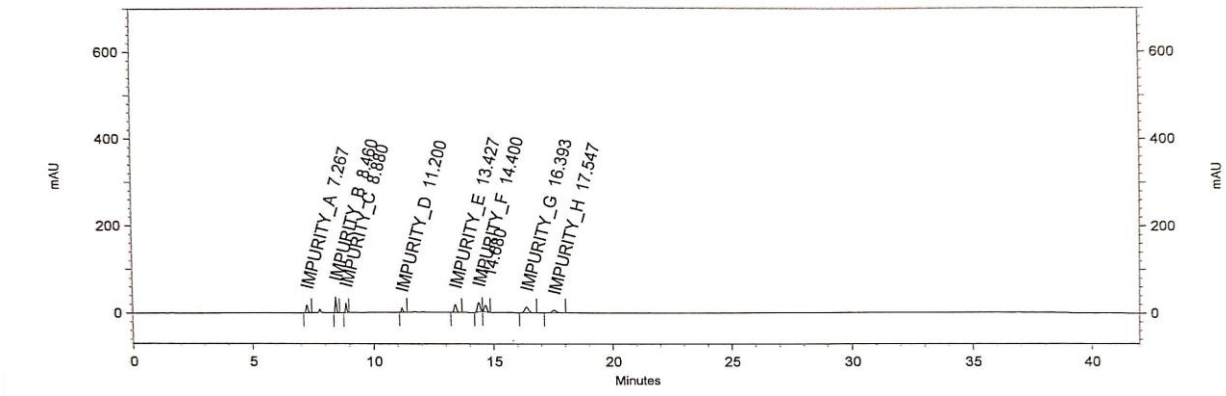

Fig No 6:Chromatogram showing all the impurities

Above chromatogram shows all the Impurities from impurity A to Impurity H which have been separated in 22 min. So we can say that our method is precise and validated.

4. Conclusion

The presented method is sensitive, precise and accurate. The advantages of the proposed method are its short analysis time and a simple procedure for sample preparation. The satisfying recoveries and low coefficient of variation confirmed the suitability of the proposed method for the routine analysis of Aceclofenac drug in pharmaceuticals dosages forms. The existing methods for determination of Aceclofenac were either costly or having more run time. The method was validated as per ICH guidelines.

Acknowledgments:
Authors are grateful to Mrs. Humera J.Khan President, Oriental Education Society, Andheri (w), Mumbai (MS) and the Principal, Oriental College of Pharmacy Sanpada, Dr.(Mrs) Sudha Rathod, for providing research facilities ,encouragement and support. Authors are thankful to Mr.Shankar karlekar and the Umedica Laboratories Pvt. Ltd., R&D center, Turbhe India for providing gift sample of Aceclofenac API and tablets.

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