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Research Article

DEVELOPMENT AND VALIDATION OF A REVERSE PHASE HPLC METHOD FOR THE DETERMINATION OF ACECLOFENAC IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, sensitive, precise, specific and accurate isocratic reversed phase-high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative estimation of aceclofenac in pharmaceutical formulations. RP-HPLC method was developed by using WELCHROM C_{18} Column (4.6 X 250mm, 5µm), SHIMADZU LC-20AT prominence liquid chromatograph. The mobile phase consisting of phosphate buffer pH 6.8 and acetonitrile in the portion of 50:50 v/v. Isocratic elution at a flow rate of 0.5 mL/min was employed. The responses are measured at 278 nm using SHIMADZU SPD-20A prominence UV-Vis detector. The retention time for aceclofenac was 8.767 min. The method possesses linearity in the range of 2-10µg/ml and correlation coefficient was 0.999. The % recovery was within the range between 99.91% and 101.26%. The accuracy and reliability of the proposed method was ascertained by evaluating various validation parameters like linearity, precision and specificity according to ICH guidelines. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The proposed method was successfully employed for routine quality control analysis of aceclofenac in bulk samples and its pharmaceutical formulations.

Keywords: Aceclofenac, Isocratic, RP-HPLC, UV-Vis detector, Method Validation.

INTRODUCTION

Aceclofenac (ACF)[1-3], 2-[2-[2-(2, 6-Dichlorophenyl) aminophenyl] acetyl] oxyacetic acid(Fig. 1) is a new phenyl acetic acid derivative with potent analgesic and anti-inflammatory propertiesand improved gastric tolerance.ACF is a non-steroidal anti-inflammatory drug (NSAID). It is a phenyl acetic acid derivative showing effective anti-inflammatory and analgesic properties, and a good tolerability profile in a variety of painful conditions. ACF has been shown to exert effects on many mediators of inflammation. It inhibits synthesis of the inflammatory cytokines, interleukin-1b and tumor necrosis factor, and inhibits prostaglandin E_2 production. A unique feature of ACF pharmacology is that it,unlike diclofenac and some other NSAIDs, stimulates glycosaminoglycans (GAG) synthesis. It is indicated for the relief of pain and inflammation in osteoarthritis, rheumatoid arthritis and ankylosing spondylitis[4-7]. Literature survey reveals thatStripping vlotametric

techniques[8],UV spectrophotometric[9-10],HPLC[11-15], Thin layer chromatography[16], Spectrofluorimetric[17], LC-MS[18] have been reported for the determination of ACF.Howeververy few HPLC methods were available for the estimation of ACF 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 ACF in dosage form and validate it, in accordance with ICH[19] guidelines.

MATERIALS AND METHODS

Chemicals and reagents

ACF was provided by Tavis life care, Delhi, India. All the chemicals were analytical grade. Potassium dihydrogen orthophosphate and phosphoric acid from Merck Specialities private Ltd., Mumbai, India.Acetonitrile (HPLC grade) and triethylamine (HPLC grade) from Merck Pharmaceuticals Private Ltd., Mumbai, India). Commercial tablets of ACF were purchased from local market.Acepac-100 tablets manufactured by Tavis life careand Flexidol-100mgtablets manufactured by Cipla.

Instrumentation

Quantitative HPLC was performed on a high pressure gradient high performance liquid chromatograph (SHIMADZU LC-20AT prominence liquid chromatograph) with two LC-20AT VP pumps, manual injector with loop volume of 20 µL (Rheodyne), programmable variable wavelength SHIMADZU SPD-20A prominence UV-Vis detector and WELCHROM C_{18} Column (4.6 X 250mm, 5µm). The HPLC system was equipped with "Spincotech" software. In addition an electronic balance (Shimadzu TX223L), digital pH meter (Systronics model 802), a sonicator (spectra lab, model UCB 40), UV- Visible Spectrophotometer (Systronics model- 2203) were used in this study.

Preparation of mobile phase

Phosphate bufferpH 6.8 was prepared by dissolving 28.80gm of disodium hydrogen phosphate and 11.45gm of potassium dihydrogen phosphate in1000mL of HPLC grade water. To this 1000mLof acetonitrile was added.The resulting solution was filtered through 0.45 µm nylon membrane filter and degassed by sonication.

Preparation of calibration standards

About 100 mg of pure ACF was accurately weighed and dissolved in 100 mL ofmobile phase to get 1 mg/mL stock solution. Working standard solution of ACFwas prepared with mobile phase. To a series of 10mL volumetric flasks, standard solutions of ACFto achieving the final concentration range of 2, 4, 6, 8, 10 µg/mL were transferred. The final volume was made with the mobile phase.

System suitability

The HPLC system was stabilized for forty min. One blank followed by six replicates of a single calibration standard solution of ACF was injected to check the system suitability. To ascertain the systems suitability for the proposed method, a number of parameters such as theoretical plates, peak asymmetry, retention time and parameters were taken and results were presented in Table 1.

Recommended procedure

Construction of calibration curve

Replicates of each calibration standard solutions (2, 4, 6, 8, 10 µg/mL) were injected into the chromatogram, the retention times and average peak areas were recorded. Calibration graph was plotted by taking concentration of ACF on X-axis and peak areas of standard ACF on Y-axis and regression equation results and relevant data were furnished in Table 2.

Analysis of marketed formulations

The content of twenty tablets was transferred into a mortar and ground to a fine powder. From this, tablet powder which is equivalent to 10 mg of ACF was taken in 10 mL of mobile phase. The resulting solution was filtered through 0.22 µm nylon membrane filter and degassed by sonication. This solution was further suitably diluted for chromatography. The test solutions were injected into the system by filling a 20 µL fixed volume loop manual injector. The chromatographic run time of 15 min. was maintained for the elution of the drug from the column. The elutes were monitored with UV detector at 278 nm. The amount of drug present in sample was computed from the calibration graph. The results were presented in Table 5. The representative standard and sample typical chromatograms showing the separation of the ACF were given in Fig. 2 and 3 respectively.

Validation study of aceclofenac

An integral part of analytical method development is validation. Once the method has been developed, it is necessary to evaluate under the expected conditions for real samples before being used for the specific purpose. The proposed method of analysis was validated as per the ICH guidelines for the parameters like specificity, precision, accuracy, linearity, robustness, system suitability, limit of detection (LOD) and limit of quantitation (LOQ).

Specificity

The effect of wide range of excipients and other additives usually present in the formulations of ACF in the determinations under optimum conditions was investigated. The specificity of the RP-HPLC method was established by injecting the mobile phase and placebo solution in triplicate and recording the chromatograms. Marketed formulations areanalyzed to determine the specificity of the optimized method in presence of common tablet excipients.

Precision

Intraday and interday precision study of ACF is carried out by estimating corresponding responses three times on the same day and on two different days for the concentration of 10µg/mL.The repeatability of sample application and measurements for peak area are expressed in terms of % RSD. The percent relative standard deviation (% RSD) is calculated which is within the acceptable criteria of not more than 2.0.

Linearity

The linearity graphs for the proposed assay methods are obtained over the concentration range of 1-10 µg/mLACF. The representative chromatograms indicating the ACFwere shown in Fig. 4 to 8. A calibration curve was plotted between concentration and area response and statistical analysis of the calibration curve was shown in Fig. 9. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient, regression data values, and the results of regression statistics were presented in Table 3 and 4 respectively.

Accuracy (Recovery studies)

The accuracy of the method is determined by calculating recovery of ACF by the method of addition. Known amount of ACF at 50%, 100%, and 150% is added to a pre quantified sample solution. The recovery studies are carried out in the tablet in triplicate each in the presence of placebo. The mean percentage recovery of ACF at each level is not less than 99% and not more than 101%.

Robustness

The Robustness is evaluated by the analysis of ACF under different experimental conditions such as making small changes in flow rate (± 0.2 ml/min), temperature (± 5^{o}C), Mobile phase composition (±5%), detection wavelength (λ_{max})and pH of the buffer solution.

Ruggedness

Ruggedness is the degree of reproducibility of results obtain by the analysis of the same sample under a variety of normal test conditions i.e., different analysts, laboratories, instruments and columns. RSD is always would be< 2%, which indicates the method is rugged.

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

Limit of detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. Limit of detection and limit of quantitation are calculated using LOD = 3.3 σ/S and LOQ = 10 σ/S formulae respectively. Where, σ = standard deviation of the peak area and S = average of the slope of the calibration curve.

RESULTS AND DISCUSSION

The mobile phase consistsof phosphate buffer pH 6.8 and acetonitrile in the ratio of 50:50 v/v. Isocratic elution was carried out with an optimized flow rate of 0.5 mL/min which gave sharp peak, and a minimum tailing factor with runtime for ACF. The retention time for ACF was 8.767 min. UV spectra of ACF showed that the drug absorbed maximum at 278 nm, so this wavelength was selected as the detection wavelength. System suitability parameters & optimized chromatographic conditions are shown in Table 1. The calibration curve for ACF was found to be linear over the range of 1-10 µg/mL. The data

of regression analysis of the calibration curve was shown in Table 2.

The developed method was applied to the assay of ACF tablets. The experimental results were given in Table 5. The representative standard and sample chromatograms of ACF were shown in Fig. 2 and 3 respectively. The regression equation was Y=124.9x + 7.309 with the correlation coefficient, r^2 =0.999 which would indicate this method had good linearity. The representative chromatograms indicating the ACF were shown in Fig. 4 to 8. The calibration plotwas shown in Fig. 9. The specificity was studied for the examination of the presence of interfering components.The comparison of chromatograms showed that there was no interference from placebo with sample peak. They did not disturb the elution or quantitation of ACF, furthermore the well-shaped peaks would also indicate the specificity of the method. Therefore, it was concluded that the method was specific. The specificity results were summarized in Table 6. Precision was studied to find out intra and inter day variations in the test methods of ACF for three times on the same day and different days. The intra-day and inter-day precision with % RSD < 2% would indicate that the proposed method was quite precise and reproducible and results were shown in Table 7. Recovery studies of the drug were carried out for the accuracy parameter at three different concentrations levels i.e. multiple level recovery studies. Known amounts of ACF standard was added into pre-analyzed sample and subjected them to the proposed HPLC method. The % recovery was found to be within the limits as listed in Table 8. Generally the mean percentage recovery of ACF at each level was not less than 99% and not more than 101%. In this case percentage recovery of ACF was found to be in the range of 99.91 to 100.26%. The method precision was done and the low %RSD 0.060values would indicate that the proposed method was in good agreement with precision. Robustness was done by small changes in the chromatographic conditions like mobile phase flow rate, temperature, mobile phase composition, detection wavelength (± 5nm) etc., It was observed that there were no marked changes in the chromatograms. Infact the parameters were within the limit, which would indicate that the method was robust and suitable for routine use. The Robustness results were presented in Table 9. The tailing factor of the peak was found to be less than 1.5 and the results were shown in Table 9. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the standard deviation of the response and the slope of the calibration curve at levels approximating the LOD and LOQ. The LOD and LOQ were found to be 0.123µg/mL, and 0.372µg/mL respectively,which would show that this method was very sensitive. The results were presented in Table 10.The validated method was applied in analysis of marketed formulations such as Acepac-100 tablets, Flexidol-100 mg tablets. The results for the assay of the drugs showed good agreement with label claims and the results were shown in Table 5.

Table 1:Optimized chromatographic conditions and system suitability parameters for proposed method

Parameter	Chromatographic conditions
Instrument	SHIMADZU LC-20AT prominence liquid chromatograph
Column	WELCHROM C_{18} Column (4.6 X 250mm, 5µm)
Detector	SHIMADZU SPD-20A prominence UV-Vis detector
Mobile phase	Buffer: ACN (50 : 50 v/v)
Flow rate	0.5mL/min.
Detection wave length	By UV at 278nm.
Run time	15 minutes
Column back pressure	65(kg/cm^2)
Temperature	Ambient temperature(25^oC)
Volume of injection loop	20µL
Retention time	8.767min
Theoretical plates[th.pl] (Efficiency)	18096
Theoretical plates per meter[t.p/m]	361914
Peak asymmetry	1.150

Table 2: Optimal chromatographic conditions,

regression data, precision of the proposed method of aceclofenac

Parameter	Method
Detection wavelength(λ max)	By UV at 278nm
Linearity range (µg/mL)	2-10µg/ml
Regression equation (Y=a+bc)	Y=124.9x+7.309
Slope(b)	124.9
Intercept(a)	7.309
Standard deviation of slope (S_m)	0.769
Standard deviation of intercept (S_c)	4.657
Standard error of estimation (Se)	0.7915
Correlation coefficient (r^2)	0.999
% Relative standard deviation* i.e., Coefficient of variation(CV)	0.060228
Limit of detection(µg/mL)	0.1230
Limit of quantitation(µg/mL)	0.372881
Percentage range of errors* (Confidence limits) 0.005significance level 0.001 significance level	 0.3071 0.0024

*Average of six determinations

Table 3: Linearity and statistical analysis data for aceclofenac

S.No.	Linearity level (µg/ml)	Peak area	Slope	Y-intercept	Correlation Coefficient(r^2)
1	2 µg/ml	262.712	124.9	7.309	0.999
2	4 µg/ml	510.585			
3	6 µg/ml	762.035			
4	8 µg/ml	1000.251			
5	10 µg/ml	1256.236			

Table 4: Regression statistics

Multiple R	0.999924207					
R Square	0.999848421					
Adjusted R Square	0.999810526					
Standard Error	6.434961191					
Observations	6					
ANOVA						
	Df	*SS*	*MS*	*F*	*Significance F*	
Regression	1	1092562.073	1092562.073	26384.82732	8.61654E-09	
Residual	4	165.6349021	41.40872553			
Total	5	1092727.708				
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	7.309333333	4.657282985	1.56944153	0.19162851	-5.621357213	20.24002
X Variable 1	124.9321	0.769124972	162.4340707	8.61654E-09	122.7966667	127.0675

Table 5: Assay results of aceclofenac formulations

S.No.	Formulations	Labeled amount (mg)	Amount found (mg)	% Assay ±RSD*
1	Acepac (Tavis life care)	100	99.98	99.98±0.11
2	Flexidol (Cipla)	100	99.97	99.97±0.12

*Average of six determinations.

Table 6: Specificity study

Name of the solution	Retention time in min.
Blank	No peaks
Aceclofenac	8.767 min.

Table 7: Results of Intraday and interday precision study

Sample	Injection number	Intraday precision	Interday precision
		Peak area	Peak area
Aceclofenac	1	1256.230	1256.410
	2	1257.231	1256.129
	3	1255.245	1256.238
	4	1256.236	1256.420
	5	1257.245	1255.235
	6	1256.212	1256.245
	Mean	1256.4	1256.113
	Standard deviation	0.752297	0.444218
	% RSD acceptance criteria 2.0)	0.060228	0.035364

Table 8: Recovery data of the proposed aceclofenac RP-HPLC method

S. No	Concentration level	Amount added (µg/mL)	Amount found (µg/mL)	Area obtained	Mean %Recovery ± SD*	%RSD #
		5	4.96	628.375		
1	50%	5	5.02	627.400	100±0.01	0.01
		5	5.04	629.260		
		10	9.98	1256.235		
2	100%	10	9.97	1256.240	99.91±0.025	0.025
		10	10.00	1256.236		
		15	15.15	1884.930		
3	150%	15	15.04	1882.124	100.26±0.05	0.05
		15	15.03	1885.870		

* SDStandard deviation.
# %RSD is percentage of relative standard deviation.

Table9: Robustness results of Aceclofenac

S. no	Parameter	Optimized	Used	Peak area	Retention time(Rt), min	Plate count	Peak asymmetry
1.	Flow rate (±0.2mL/min)	0.5 mL/min	0.3 mL/min	1284.875	8.991	17629	1.194
			0.5 mL/min	1256.236	8.767	18096	1.150
			0.7 mL/min	1232.754	8.650	17547	1.074
2.	Detection wavelength (±5nm)	278nm	273nm	1238.620	8.767	18096	1.150
			278nm	1256.236	8.767	18096	1.150
			283nm	1240.540	8.767	18096	1.150
3.	Mobile phase composition (±5%)	50:50v/v	55:45v/v	1230.250	7.990	17987	1.182
			50:50v/v	1256.230	8.767	18096	1.150
			45:55v/v	1228.786	9.627	18998	1.167

Table 10: Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection (LOD)	0.1230µg/mL
Limit of Quantitation (LOQ)	0.3728µg/ml

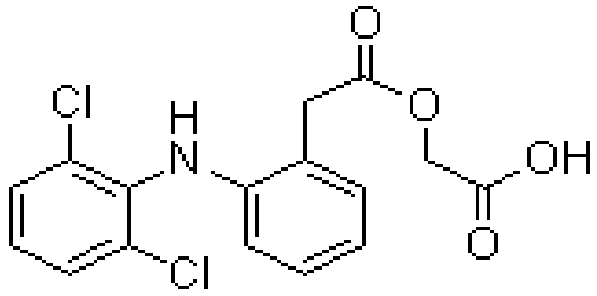

Fig. 1: Chemical name and structure of Aceclofenac

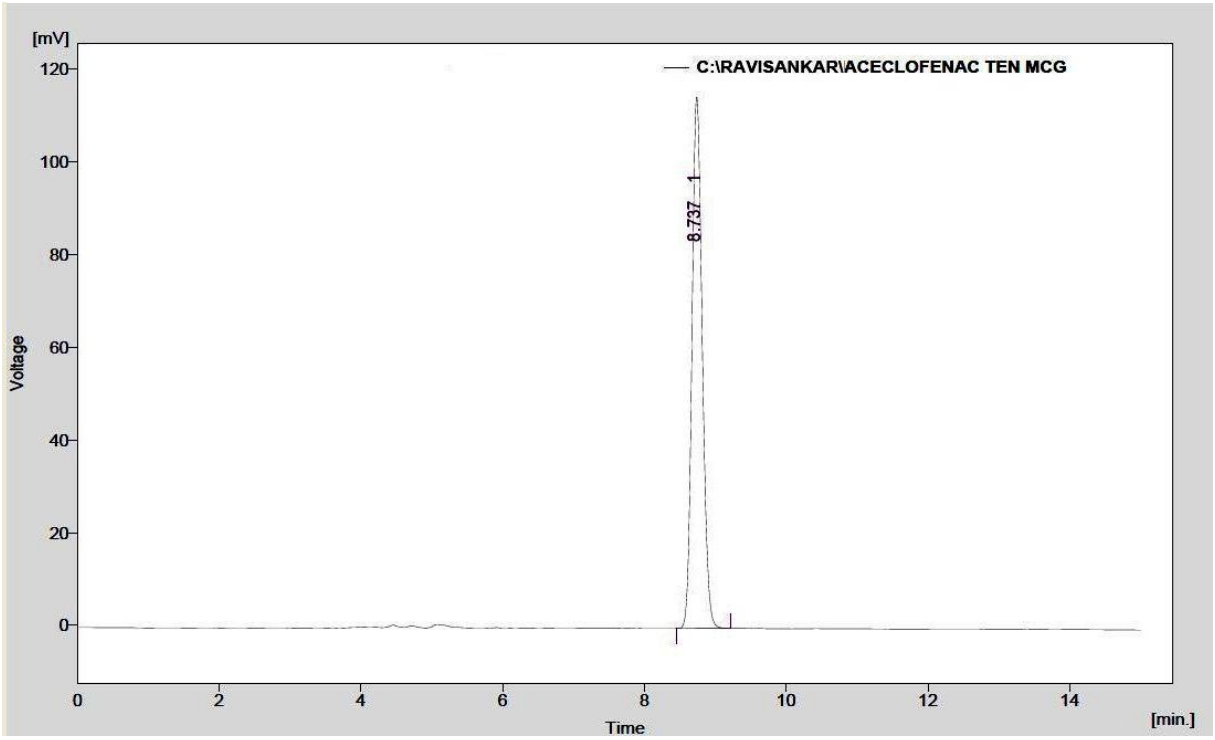

Fig. 2: Standard chromatogram of aceclofenac (10µg/mL)

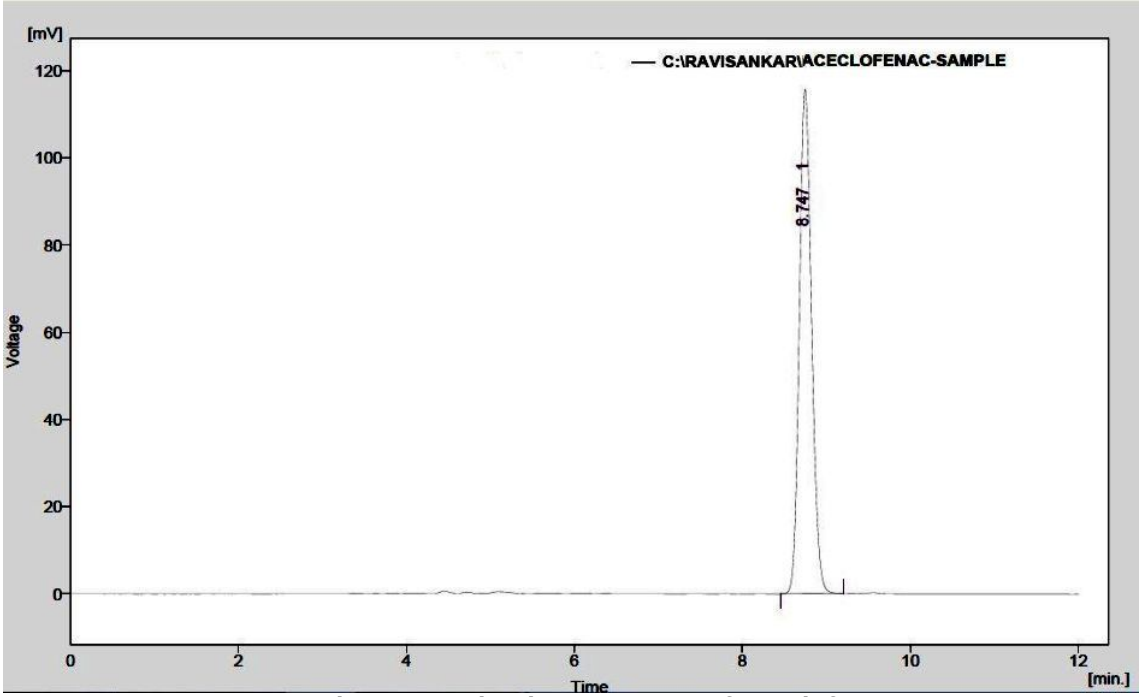

Fig. 3: Sample Chromatogram of Aceclofenac

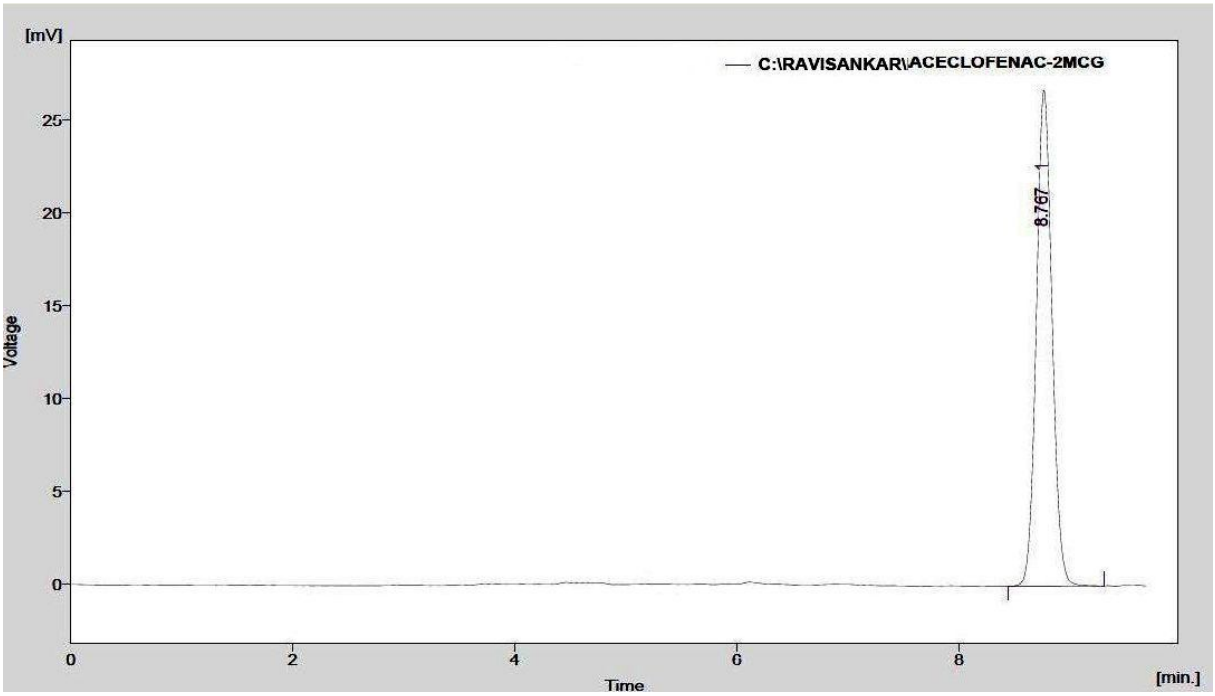

Fig. 4: Standard chromatogram of aceclofenac(2 µg/mL)

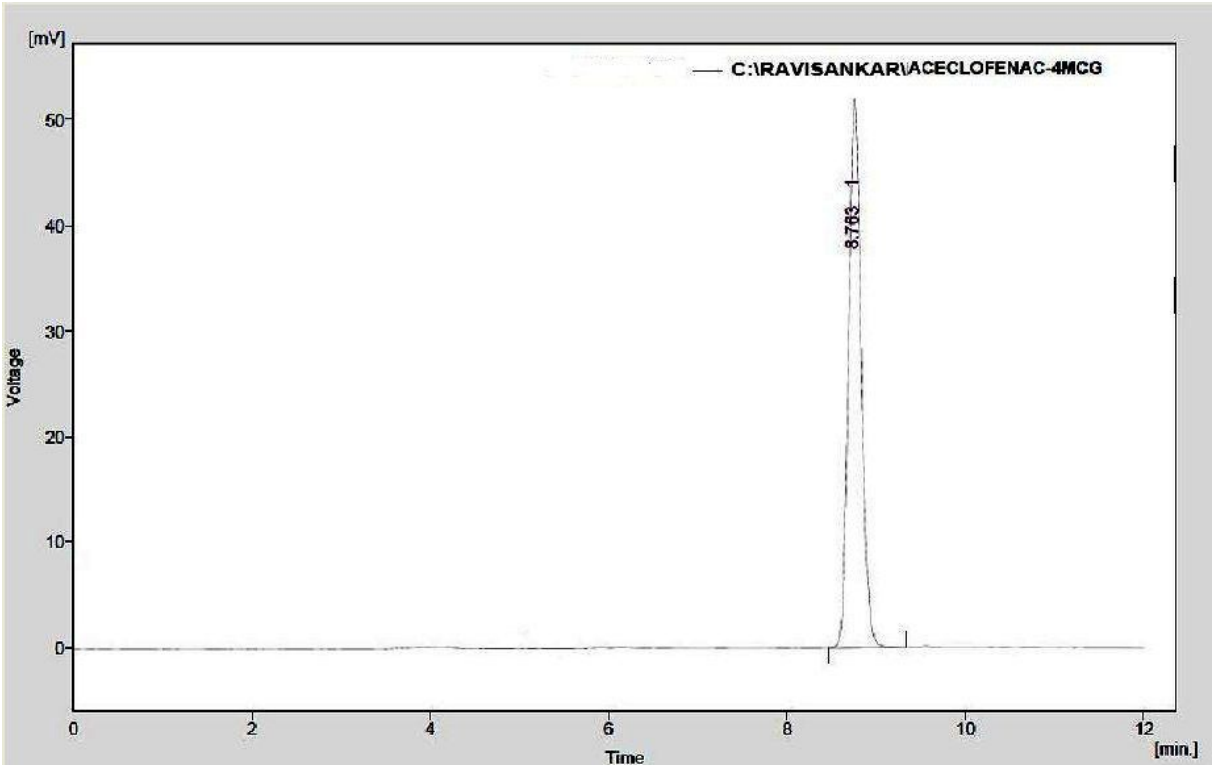

Fig. 5: Standard chromatogram of aceclofenac (4µg/mL)

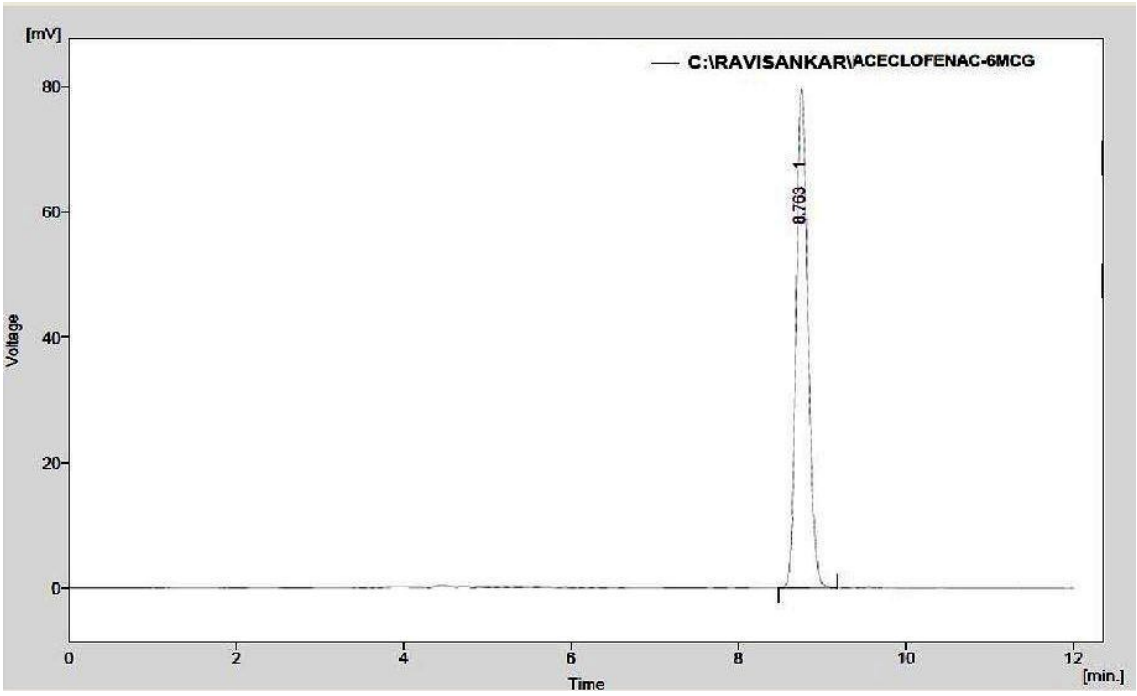

Fig. 6: Standard chromatogram of aceclofenac(6 µg/mL)

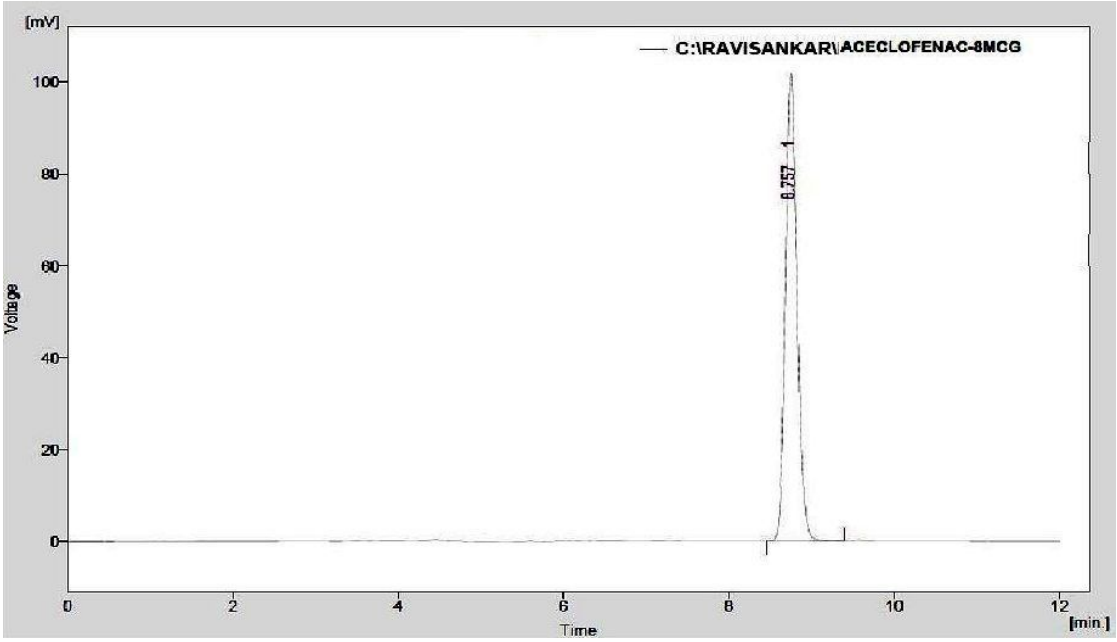

Fig. 7: Standard chromatogram of aceclofenac(8 µg/mL)

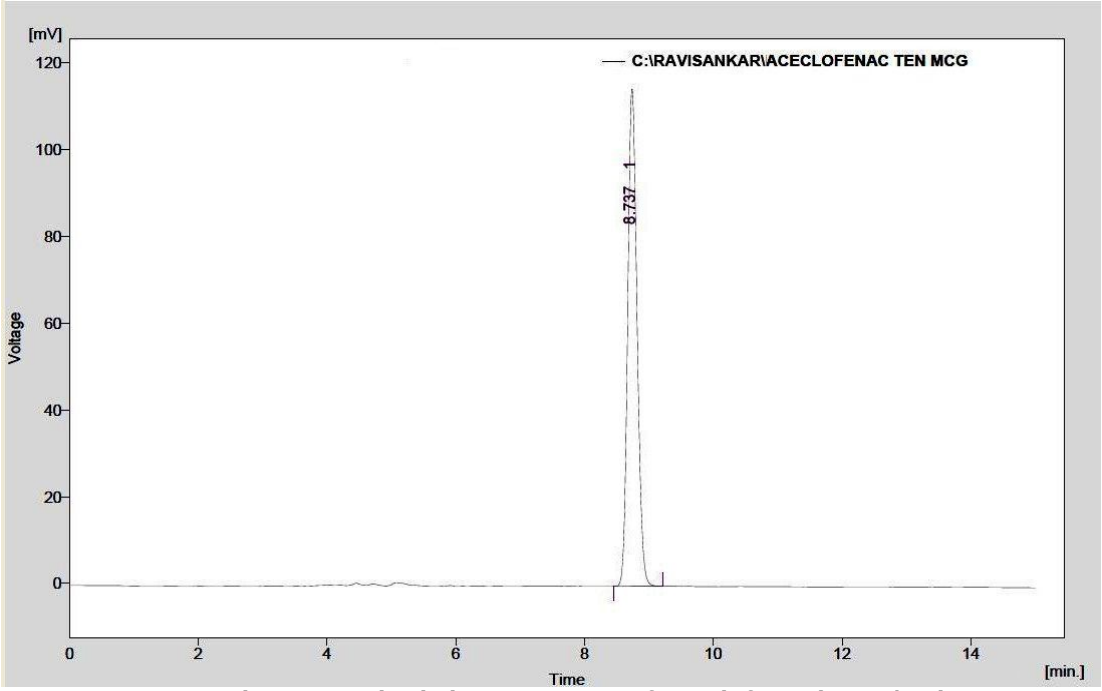

Fig. 8: Standard chromatogram of aceclofenac(10 µg/mL)

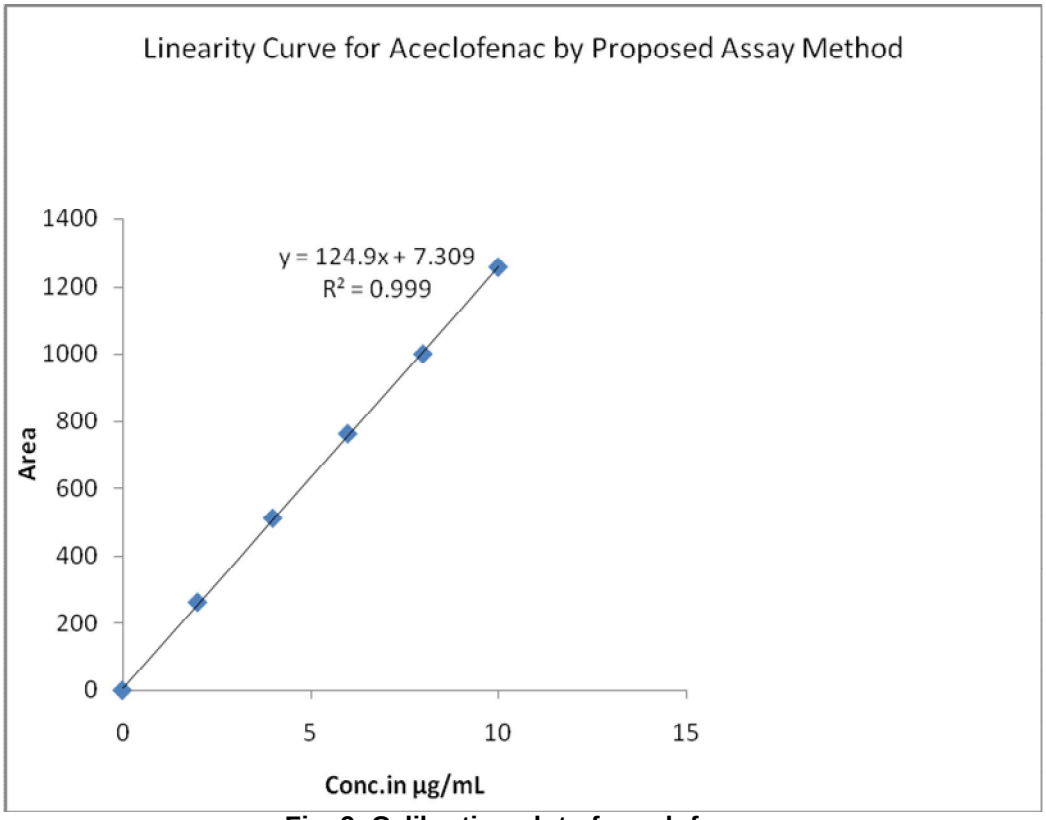

Fig. 9: Calibration plot of aceclofenac

CONCLUSION

A validated RP-HPLC method has been developed for determination of aceclofenac in bulk and tablet dosage forms. Statistical analysis of the results shows that the proposed procedure has good precision and accuracy. Results of analysis of pharmaceutical formulations reveal that the proposed method was suitable for their analysis with virtually no interference of the usual additives presented in pharmaceutical formulations. This method is simple, reliable, accurate, linear, sensitive, economical and reproducible. Hence this method can be suitable for routine quality control analysis of ACF in active pharmaceutical ingredient (API) and pharmaceutical preparations.

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