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RP-HPLC Method Development and Validation for the Analysis of Pharmaceutical Drug – ACECLOFENAC

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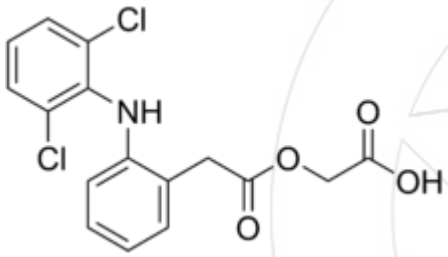
Abstract: ***A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of ACECLOFENAC. Isocratic elution at a flow rate of 1.2 ml /min was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of Acetonitrile: 0.1M Acetic Acid 60:40 (v/v). The UV detection wavelength was at 210 nm. Linearity was observed in concentration range of 0.01-0.05 gm/ml. The retention time for ACECLOFENAC was 3.0 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of ACECLOENAC.***

Keywords: Aceclofenac, Method Development, Validation, 210nm, 3.0min.

1. Introduction

DRUGS: Aceclofenac

Structure



IUPAC Name	[[[2-[(2,6-Dichlorophenyl)amino]phenyl] acetyl]oxy]acetic acid
Formula	$C_{16}H_{13}Cl_2NO_4$
Molacular Weight	353.03 g/mol
Density	1.455 g/cm³
Melting Point	149 - 153^0 C [2]
Solubility	Practically insoluble in water, freely soluble in acetone, soluble in alcohol.

Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID) analog of Diclofenac. It is used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis.

It should not be given to people with porphyria or breast-feeding mothers, and is not recommended for children. It should be avoided near term in a pregnant woman because of the risk of having a patent ductus arteriosus in the neonate.

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. The incidence of gastric ulcerogenicity of aceclofenac has been reported to be significantly lower than that of the other frequently prescribed NSAIDs, for instance, 2-folds lesser than naproxen, 4-folds lesser than diclofenac, and 7-folds lesser than indomethacin. Aceclofenac (C16H13Cl2NO4), chemically [(2-{2, 6-dichlorophenyl) amino} phenylacetooxyacetic acid], is a crystalline powder with a molecular weight of 354.19. It is practically insoluble in water with good permeability. It is metabolized in human hepatocytes and human microsomes to form [2-(2',6'-dichloro-4'-hydroxy- phenylamino) phenyl] acetoxyacetic acid as the major metabolite, which is then further conjugated. According to the Biopharmaceutical Classification System (BCS) drug substances are classified to four classes upon their solubility and permeability. Aceclofenac falls under the BCS Class II, poorly soluble and highly permeable drug. [1]

2. Experimental

Chemicals and reagents

All HPLC SOLVENTS used like Acetonitrile, Acetic Acid which are of HPLC grade were purchased from E.Merck,

Instrumentation and analytical conditions

The analysis of the drug was carried out on Shimadzu HPLC model (VP series) containing LC-20AT (VP series) pump, variable wave length programmable UV/visible detector SPD-20A and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using phenolex C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing. Isocratic elution with, Acetonitrile, 0.1MAcetic Acid 60:40(v/v) was selected with a flow rate of 1.2 ml /min .The detection wavelength was set at 210 nm with a run time of 10 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. The column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

Preparation of Stock, working standard solutions and Sample solutions

100 mg of Aceclofenac was weighted and transferred into a 100 ml volumetric flask. Water was added and sonicated to dissolve it completely and made up to the mark with the same solvent. Further 10 ml of the above stock solution was pipette into a 100ml volumetric flask and diluted up to the mark with water. The contents were mixed well and filtered through Ultipor N66Nylon 6, 6 membrane sample filter paper. The calibration curve was plotted with the concentrations of the 0.01-0.05 gm/ml solutions. Calibration solutions were prepared and analyzed immediately after preparation.

Table 1: Chromatographic conditions for Aceclofenac

S. No	Test H.P.L.C Conditions	Result
1	Elution	ISOCRATIC
2	A.P.I Conc.	0.01 gm/ml
3	Mobile Phase	Acetonitrile:0.1M Acetic Acid(60:40)
4	pH	3.2
5	Column	C18
6	Wavelength	210 nm
7	Flow Rate	1.2ml/min
8	Runtime	10 Min
9	Retention Time	3.0
10	Area	600.05
11	Th.Plates	4950
12	Tailing Factor	1.007
13	Pump Presure	105 kgf

Method Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, robustness and system suitability.

Linearity

Table 2: Linearity of Aceclofenac

S.NO	CONC	AREA
1	0.01 gm/ml	600.050
2	0.02 gm/ml	1232.876
3	0.03 gm/ml	1890.788
4	0.04 gm/ml	2499.123
5	0.05 gm/ml	3099.006

The developed method has been validated as per ICH guidelines.Solutions of Aceclofenac in the mass concentration range of 0.01 gm/ml to 0.05gm/ml was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of Aceclofenac was obtained by plotting the peak area ratio versus the applied concentrations of Aceclofenac. The linear correlation coefficient was found to be 0.9998

Table 3: Linear Regression Data for Calibration curve

Drug	Paracetamol
Concentration range	0.01 - 0.05gm/ml
Slope (m)	63312.15
Intercept (b)	-32.41
Correlation coefficient	0.9998

Precision

Repeatability of the method was checked by injecting replicate injections of 0.01 gm/ml of the solution for five times on the same day as intraday precision study of and the RSD was found to be 0.1238 for intraday and 0.1162 for interday

Table 4: Precision parameters of Aceclofenac

Injection	*Concentration*	*Intra Day*	*Inter Day*
1	0.01 gm/ml	599.752	603.105
2	0.01 gm/ml	599.133	604.099
3	0.01 gm/ml	599.990	603.433
4	0.01 gm/ml	600.254	605.072
5	0.01 gm/ml	600.585	604.272
6	0.01 gm/ml	600.802	604.089
	%RSD	0.1005	0.1005

Accuracy

The accuracy of the method was determined by calculating recovery of Aceclofenac by the method of standard addition. Known amount of Aceclofenac (0.01) was added to a pre-quantified sample solution and the amount of Aceclofenacl was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Aceclofenac was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve.

Specificity

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of test results those obtained from standard drug.

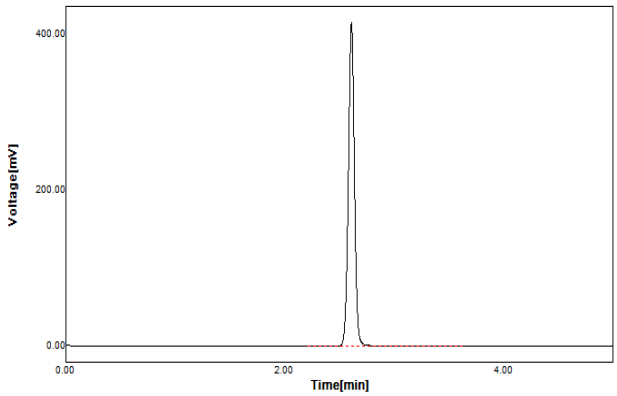


Figure: Typical chromatogram of Aceclofenac

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 1ppm and 10 ppm respectively as per ICH guide-lines. Results are shown in table 5.

Table 5: Results of LOD and LOQ.

Parameter	Measured
LOD	0.001gm/ml
LOQ	0.01 gm/ml

Robustness

To determine the robustness of the method, two parameters from the optimized chromatographic, Conditions were varied. First, Instrument and place were changed and second mobile phase concentration was changed 0.1M to 0.2M, 0.05M. Results of Robustness are shown in table 6& 7.

Table 6: Robustness parameters

Parameter	*Modification*
M.PHASE	Acetonitrile:0.05M Acetic Acid(60:40)
PH	3.5
WAVELENGTH	210 nm
R.T	2.503 Min

Table 7: Robustness results

Accuracy	*Precision*
570.133	567.244
573.133	566.999
573.159	567.230
	566.847
	566.990
RSD: 0.304	RSD: 0.032

System Suitability Parameter

System suitability tests were carried out on freshly prepared standard stock solutions of Aceclofenac and it was calculated by determining the standard deviation of Aceclofenac standards by injecting standards in five replicates at 5 minutes interval and the values were recorded in Table 8.

Table 8: System suitability parameters of Aceclofenac

Parameters	*Values*
λ max (nm)	210 nm
Correlation coefficient	0.9997
Retention time	2.850min
Theoretical plates	4950
Tailing factor	1.009
Limit of detection	0.001 gm/ml
Limit of quantification	0.01 gm/ml

3. Result and Discussion

Optimization of the chromatographic conditions:

The nature of the sample, its molecular weight and solubility decides the proper selection of thestationary phase. The drug Aceclofenac being non-polar is preferably analyzed by reverse phase columns and accordingly C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Acetonitrile: 0.1M Acetic Acid (60:40).The retention time of Aceclofenac was found to be 3.0 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise.

4. Conclusion

A validated RP-HPLC method has been developed for the determination of Aceclofenac in bulk form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 6 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Aceclofenac in pharmaceutical analysis.

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