

METHOD DEVELOPMENT AND VALIDATION OF ACECLOFENAC BY USING RP-HPLC

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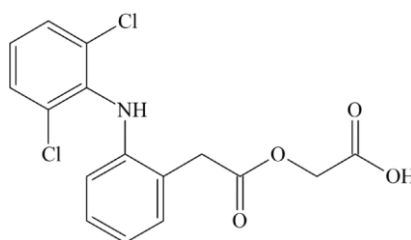
ABSTRACT

This study presents the development and validation of a high performance liquid chromatography (HPLC) method for the analysis of Aceclofenac. The chromatography condition were optimized, and the method was validated for specificity, precision, accuracy, linearity, and robustness. Result indicate that the developed HPLC method is suitable for accurate quantification of Aceclofenac in pharmaceutical formulation. This research contributes to ensuring the quality and reliability of aceclofenac analysis in pharmaceutical laboratories.

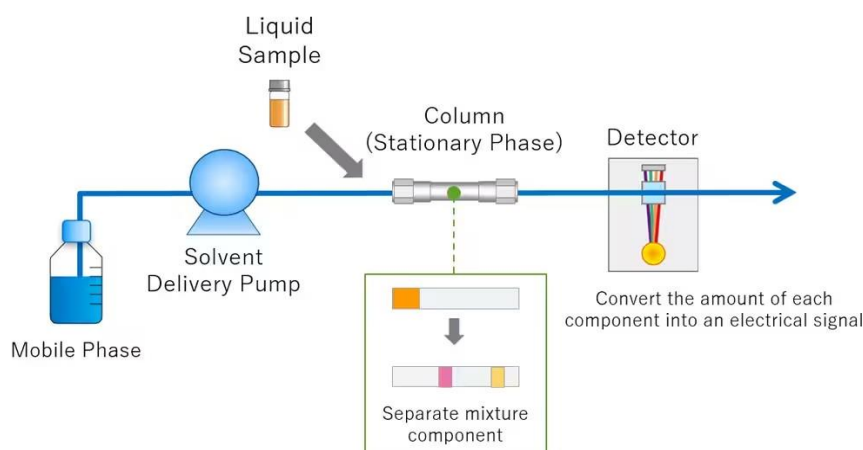
Keywords: Aceclofenac, Methanol, Liquid Chromatography, HPLC Method.

I. INTRODUCTION

Analysis is vital in any product to service, and it's also important in drug because it involve life. Analytical chemistry is the analysis of separation, quantification and chemical additives identification of herbal and synthetic materials constituted with one or more compounds or factors. ¹To quantify and evaluate the products, an analytical method needs to be validated. Validation establishes the pathways for the capability of methods.² Validations shall be performed for spectroscopic methods, Isolation methods, Separation methods and Characterization methods.³ Chromatographic method working on nature of the molecules such as chemical and physical properties. The primary factors are appropriate separation, retention and efficiency. System suitability is the major parameter for finalization of method conditions.



Structure of aceclofenac



Molecular weight: 354.18 g/mol, Molecular formula: C₁₆H₁₃Cl₂N₂O₄ IUPAC Name: 2- [2- [2-(2,6-dichloroanilino) phenyl acetyl] oxyacetic acid, Melting Point: Standard value-149-1530C Observed value-1510C Category:⁴ Indicated for the relief of pain and inflammation in osteoarthritis, rheumatoid arthritis and ankylosing spondylitis.

II. MATERIALS AND METHOD

Materials

Procurement of the drugs specimens draws atmost priority. Aceclofenac Active Pharmaceutical Ingredient was obtained from pharma labs.

Reagents & Chemicals

Methanol (HPLC grade), Acetonitrile (HPLC grade), Water (HPLC grade), Glacial acetic acid (LOBA Chemicals)

Instruments used

Different instrument used to carry out the work are Shimadzu AUX-220 Digital balance. Shimadzu LC-2010CHT, Systronics pH meter, upH system 361, Shimadzu UV 160A recording spectrophotometer, Sonicator- Sonica Ultrasonic Cleaner-Model 2200 MH, Shimadzu LC 10 Atvp solvent delivery system , Rheodyne 7725i with 20ul loop, Class VP data system

The separation in the RP-HPLC is based on the polar nature of the drug and the polarity of the mobile phase. The column used in usually a C8 or C18column, which are less polar than the mobile phase.

METHODOLOGY

Selection of detection Wavelength

Solutions Aceclofenac (10 µg/ml) were prepared in the mobile phase and scanned in the UV region of 200 – 400 nm and recorded the spectrums. From the overlain spectrum was found that drug have marked absorbance at 270nm and can be effectively used for estimation of drug without interference. Therefor 270nm was selected as detection wavelength for estimation the drug by RP- HPLC method with an Gradient elution technique.

PREPARATION OF STANDARD SOLUTION

Accurately weighed 20mg of Aceclofenac were taken and transferred into a 10ml clean volumetric flask. The drug Were dissolved in methanol and made upto the volume with methanol to obtain 2000µg of Aceclofenac. Then pipette 2.5ml of above solution and transferred into 25ml volumetric flask the final concentration obtained was 200µg of Aceclofenac.

PREPARATION OF SAMPLE SOLUTION

For analysis of tablet dosage form, tablet containing 200mg of Aceclofenac, were weighed and their average weight was calculated. The tablets were finely powdered and powder equivalent 200mg Aceclofenac were accurately weighed and dissolved in 100 mL of methanol. The solution was sonicated for 30 min, filtered through the Whatman No. 41 filter paper and the residue was washed with methanol. This solution was further diluted with methanol to get the same concentration as that of the final standard solution.

Optimization of chromatographic condition:

The initial chromatographic conditions used for the elution of Aceclofenac.

Mode of operation	- Gradient
Stationary phase	- C18 column (250mm×4.6mm i.d.5µ)
Mobile phase	- Acectronitrile: Methanol: Water
Ratio	- 50:20:30%v/v/v
Detection wavelength	- 270nm
Flow rate	- 1.2ml/min
Temperature	- 35°C
Sample load	- 20µL
Method	- External standard calibration method

III. METHOD OF ANALYSIS

With optimized chromatographic condition baseline was recorded after the stabilization of the baseline for about 20 min. Successfully volume of the standard solution was injected and chromatogram was recorded, until the reproducibility of the peak areas were satisfactory. This procedure was followed for sample test solution such that duplicate injection of sample test solution were bracketed by injection of standard solution.

Selection of mobile phase

Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded, they include the following.

TABLE: 1

S. No.	Mobile phase	Observation
1	Methanol: Acetonitrile (50:50%v/v)	First peak show Fronting
2	Acetonitrile: Methanol: Water (50:20:30%v/v/)	First and second peaks merged
3	Acetonitrile: Water: Glacial acetic acid 2% (85:10:5v/v/v) with adjusted pH7.5 using acetic acid	Drug were eluted with sharp peaks and better resolution.

From the above information, in the mobile phase of Acetonitrile: Water: Glacial acetic acid with adjusted pH 7.5 using acetic acid (85:10:5 v/v/v), these drug were eluted with sharp peak and better resolution. Hence this mobile phase was used.

Validation method:

System suitability studies

The system suitability studies were carried out as specified in I.P. the parameter like Column efficiency, Tailing factor, Asymmetric factor, and Theoretical plate number and were calculated.

Linearity

A calibration curve was plotted between concentration versus the peak area. The linearity range was checked for in the concentration range of 20-100 µg of Aceclofenac. The drug was found to be linear in the specified concentration range

Precision

The repeatability of the method was checked by repeated analysis of the formulation for six times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The percentage RSD was calculated.

Accuracy

Accuracy of the method was confirmed by recovery studies. To the 50% pre-analyzed formulation, a known quantity of the standard drug solution was added and the amount of drug recovered was calculated. The %RSD was calculated.

LOD and LOQ

The linearity study was carried out for three times. The LOD and LOQ were Calculated based upon the calibration curve method. The LOD and LOQ were calculated by using the average of slope and standard deviation of response.

IV. RESULT AND DISCUSSION

RP - HPLC METHOD

A simple, rapid, accurate and precise method for the estimation of Aceclofenac in pure and tablet dosage form by an Gradient RP - HPLC method.

A 10µg/ml solution of Aceclofenac were prepared in the diluent Methanol. The solutions were scanned between 200 to 400 nm and the spectra were recorded. Aceclofenac have marked absorbance at 270 nm and hence it was selected as the detection wavelength. The drug Aceclofenac was found to be stable for 2 hour respectively

UV SPECTRUM:

Determination of λ max

The λ max of aceclofenac of was found to be 270 nm in methanol.

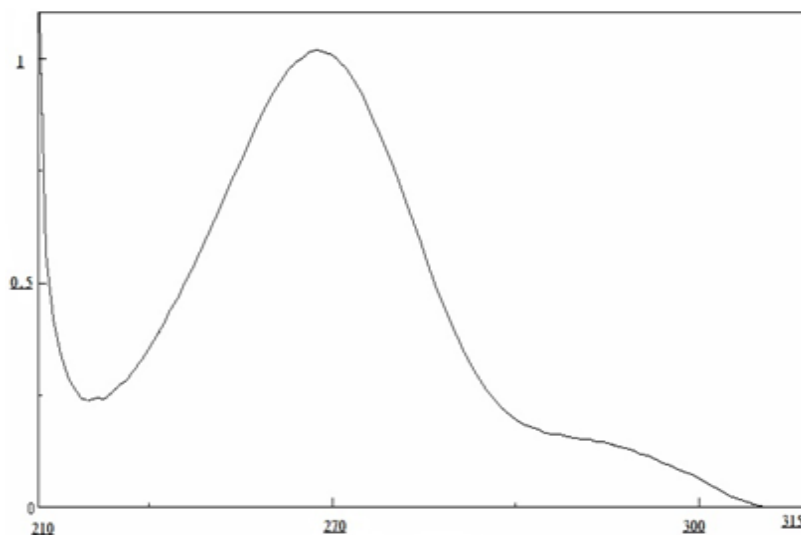


FIG: 1

LINEARITY AND CALIBRATION

From the standard solution, pipette out 1-5 ml onto a series of six 10 ml volumetric flask and made upto the mark with mobile phase to obtain the concentration 20-100 μ g of Aceclofenac solution were injected and chromatogram was recorded. The calibration curve was plotted between concentration verses peak area.

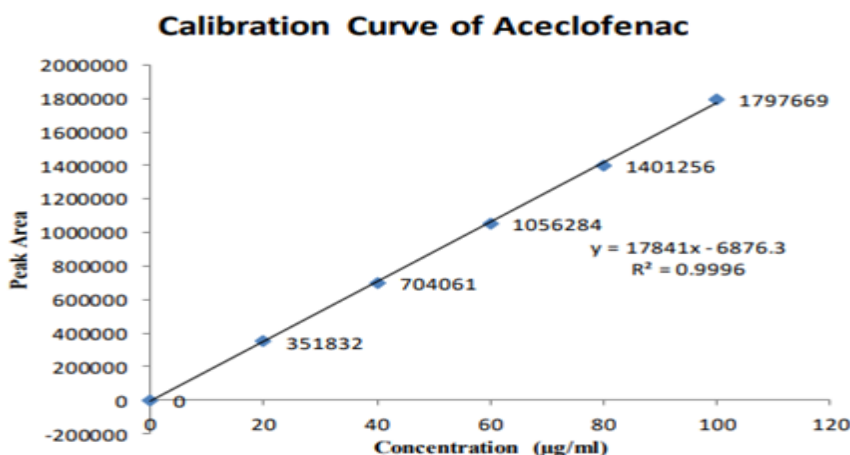


FIG: 2

QUANTIFICATION OF FORMULATION

Estimation of Aceclofenac in tablet formulation by RP-HPLC was carried out using optimized chromatographic conditions. Ten tablet of formulation were weighed accurately. The average weight of each tablet was found and powdered. Weighed accurately the tablets equivalent to 200mg of Aceclofenac in a 100ml Volumetric flask and dissolved in methanol, and made upto the mark with same(2000 μ g/ml). From the clear solution, further dilution made by diluting 1ml into 10ml volumetric flask, and further transferred 2ml into 10ml to obtain 40 μ g/ml of Aceclofenac and 30 μ g/ml theoretically. The peak area measurements were done by injecting sample six times and the amount of Aceclofenac concentration were calculated from their respective calibration curve.

ACCURACY

TABLE: 2

Drug	Sample no.	Labelled amount (mg / tab)	Amount Found (mg / tab)	Percentage Obtained	Average	S.D	%R.S.D
ACF	1	200	200.17	100.08	100.59	0.3629	0.3607
	2	200	202.03	101.01			
	3	200	200.82	100.41			
	4	200	201.86	100.93			
	5	200	201.51	100.75			
	6	200	200.75	100.37			

Accuracy of the method was determined by replicates (n=3) analysis, carried out using three solutions prepared by standard addition of pure active pharmaceutical ingredient at three different concentration levels of 80%, 100% and 120%. Accuracy was calculated by comparing the difference between the spiked value (theoretical value) and that actual found value. Results are presented in the term of %recovery of the active pharmaceutical ingredient. Each concentration was repeated for three times.

TABLE: 3

Drug	Percentage %	Amount added (µg/ml)	Amount estimated (µg/ml)	%Recovery	S.D	%R.S.D
ACF	80	48	47.76	99.50	0.5074	0.5094
	100	60	59.49	99.15		
	120	72	72.11	100.15		

Preparation of calibration curve for the serial dilution of standard was repeated for six times. The limit of detection and limit of quantification of each were calculated by using the average value of slope and standard deviation of response (Intercept).

TABLE: 4

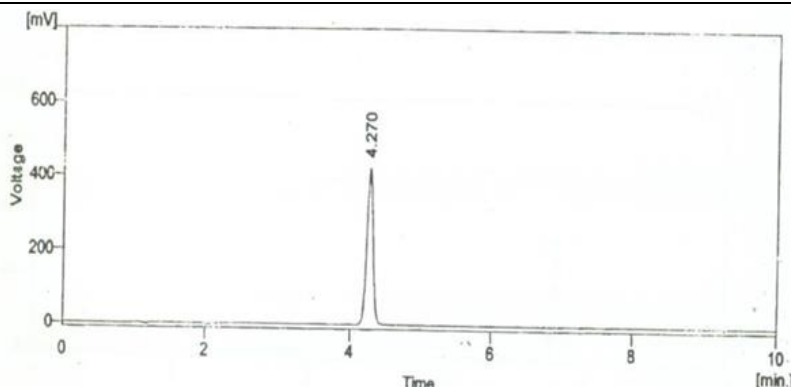
Parameters	Aceclofenac
Concentration (µg/ ml)	20-100
Correlation coefficient (r)	0.999
Regression equation (y=mx+c)	Y=17841x-6876
Slope (m)	17841
Intercept (C)	-6876
LOD (µg/ ml)	0.0006
LOQ (µg/ ml)	0.0245

SYSTEM SUITABILITY:

To verify whether the analytical system is working properly or it can give accurate and precise results, the system suitability parameters are to be set. Inject separately 20 L each of the following solutions into the HPLC.

TABLE: 5

System suitability parameters	Aceclofenac
Tailing factor	1.1 [NMT 2.0]
Number of theoretical plate	2654 [NLT 2000]



PRECISION

Precision is the measure of the degree of repeatability of an analyte method under normal operation and is normally expressed as percent relative standard deviation for a significant number of the samples.

According to the ICH precision should be performed at three different levels: Repeatability, Intermediate precision, Reproducibility.

V. CONCLUSION

RP-HPLC method was developed. It was validated for the estimation of Aceclofenac tablet dosage form using HPLC Shimadzu Shimadzu UV 160A recording spectrophotometer, C18 column (250x4.6mm, 5 μ) injection of 20 μ l is injected and eluted with the mobile phase of Acetonitrile : Methanol : Water Ratio 50:20:30%v/v/v, which was pumped at a flow rate of 1.2ml/min. The calibration curve was plotted with peak area versus concentration and the correlation coefficient was found to be 0.999 for Aceclofenac respectively. The developed method was validated for various parameters as per ICH guidelines like Accuracy, Precision, Linearity, Specificity, Ruggedness, Robustness, LOQ and LOD.

The analytical method validation of Aceclofenac drug by RP HPLC method was found to be satisfactory and could be used for the routine pharmaceutical analysis of Aceclofenac drug.

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VI. REFERENCE

- [1] Hema, Swati Reddy G. et al., A review on new analytical method development and validation by RP-HPLC. *Int Res J Pharm Biosci* 2017;4:41-50.
- [2] Ravisankar P, Navya CN, Pravallika D, Sri DN. et al., A review on step-by-step analytical method validation. *IOSR J Pharm* 2015;5:7-19.
- [3] Patel A, Dwivedi N, Kaurav N, Bashani S, Patel S, Sharma HS, et al. Chemical analysis of pharmaceuticals: a review. *J Med Pharm Innov* 2016;3:4-7.
- [4] Kakasaheb Ramoo Mahadik, et al., 2012, Reported the "Application of HPLC for the Simultaneous Determination of Aceclofenac, Paracetamol and Tramadol Hydrochloride in Pharmaceutical Dosage Form".
- [5] Rohit Shah*, Chandrakant Magdum, Shital Kumar Patil, KR Gupta V. et al., Validated Spectroscopic Method for Estimation of Aceclofenac from Tablet Formulation *Research J. Pharm. and Tech.*(2008), (4): 430-432.
- [6] Paresh M, Bhanudas K, et al., Simultaneous Spectrophotometric Estimation of Aceclofenac and Paracetamol in Tablet Dosage Form *Indian Journal of Pharmaceutical Sciences* (2010), (1):24-29.
- [7] Pawar V.T., Pishawikar S.A., More H.N., et al., Spectroscopic Estimation of Aceclofenac & Paracetamol tablet dosage form *International Research Journal of Pharmacy* (2011), (2): 93-98.