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RP-HPLC METHOD FOR THE ESTIMATION OF ACECLOFENAC IN TABLET DOSAGE FORM

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ABSTRACT

A simple, sensitive and precise RP-HPLC method was developed for the determination of aceclofenac in tablet dosage form. The RP-HPLC separation was achieved on hypersil C18 column (250 mm, 4.6 mm, 5µm) using mobile phase water: acetonitrile (55:45 v/v) at flow rate of 1 ml/min at ambient temperature. Quantification was achieved with photodiode array detection at 277 nm over the concentration range of 1-10 µm/ml. The method was validated statistically and applied successfully for the determination of aceclofenac.

Keywords: LC-MS method, HPLC method, Aceclofenac, Tablet dosage form, Nonselective cyclooxygenase inhibitor, Anti-inflammatory agent.

INTRODUCTION

Aceclofenac is nonselective cyclooxygenase inhibitor that is anti-inflammatory agent used for inflammation, headache, gout, rheumatoid arthritis. Aceclofenac is chemically [({2-[(2,6-dichlorophenyl)amino]phenyl}acetyl)oxy]acetic acid with an empirical formula $C_{16}H_{13}Cl_2NO_4$, representing molecular weight of 354.18g/mol figure2.[1] Literature survey revealed LC-MS and HPLC methods for estimation of Aceclofenac in human plasma and pharmaceutical dosage forms.[2-7] So it was thought of interest to develop a simple, specific and sensitive RP-HPLC method for determination of aceclofenac.

MATERIALS AND METHODS

All the reagents used were of HPLC grade and analytical grade. Reference standard of aceclofenac was supplied as gift sample from Sun Pharmaceutical Laboratories Limited, Jammu with purity of 99.987% tablets of three different companies (ACLOFEN-100 mg from M/s Ind. Swift Pharma Ltd., HIFENAC-150 mg from M/s Intas Pharma Ltd. and ACIFON-200 mg from M/s Zenon Pharma Ltd) were procured from the local pharmacy in the market figure3. A standard stock solution of Aceclofenac (1 mg/ml) was prepared by dissolving 25 mg of drug in 25 ml of acetonitrile. [9-12] Working standard solution (100 µm/ml) was prepared from stock solution by proper dilution with acetonitrile and water mixture. A Younglin (LC - 2010TH –Liquid Chromatograph) equipped with PDA detector. Promesil C18 ((250 mm, 4.6 mm, 5µm) column and LC software were used.[13] The mobile phase used was water: acetonitrile (55:45 v/v) which was filtered through nylon 0.45 µm

membrane filter and degassed by ultrasonication

RESULTS AND DISCUSSION

Linearity of the method was investigated by serially diluting the working standard to give a concentration range of 1-10 µm/ml and 20 µl from this was injected. The flow rate was maintained at 1 ml/min. temperature of column was kept ambient and the effluent was monitored at 277 nm. Calibration curve (figure4) was constructed by plotting concentration against peak area. The method was validated for linearity, precision, accuracy, specificity, limit of detection and limit of quantification as per ICH guidelines.

Assay of tablets of Aceclofenac were performed (table4). Twenty tablets of each company of strength 100 mg, 150 mg and 200mg were weighed and ground to a fine powder. A quantity of tablet powder equivalent to 10 mg of Aceclofenac was transferred to 10 ml volumetric flask, dissolved and diluted with acetonitrile and water mixture to obtain 1 mg/ml. The solution was sonicated for 15 minute and filtered through 0.45 µm membrane filter. The solution was further diluted to obtain concentration 10 µm/ml. Peak area of the above prepared tablet solutions of aceclofenac were measured by using above mentioned chromatographic conditions and the amount of aceclofenac were found from regression equation.

To optimize the HPLC parameters, several mobile phase compositions were tried. Various mobile phases having different ratios of methanol, water and acetonitrile were tried. Drug was retained in mobile phase consisting of methanol : water (60 : 40 v/v) and methanol : acetonitrile (55 : 45 v/v). In methanol : water (60 : 40 v/v) tailing in the peak was observed. Good peak symmetry and satisfactory retention time was obtained with mobile phase consisting of water : acetonitrile (55:45 v/v). Quantification was achieved with PDA detection at 277 nm based on peak area. The retention time of aceclofenac obtained was 6.60±0.132 (figure1). for 15 min (table 1).[14,15]

The system suitability tests for HPLC were carried out on freshly prepared solution of Aceclofenac (10 µg/ml) and parameters were studied. The results were summarized in Table4. The linear regression date showed a good linear relationship over the concentration range of 1-10 µg/ml as summarized in (table3). The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were found by scanning the solution of aceclofenac having different lower concentrations and the LOD and LOQ were found to be 0.5 and 1 µg/ml indicates that method is sensitive (table3) The intraday and interday precision were determined by analyzing standard solution of aceclofenac at three different concentration levels (6, 8, 10 µg/ml). The % RSD for intraday and interday precision was found to be 0.257 – 0.712% and 0.480-1.080% respectively which indicate that method is precise (table3) Repeatability of the method was studied by injecting 10 µg/ml solution of aceclofenac for six times and peak area was measured and % RSD was calculated which was found to be 0.195 shows repeatability of the method (table3) Accuracy of the method was evaluated by standard addition method in which appropriate portion of stock solutions of Aceclofenac were spiked into blank placebo matrix to produce concentrations of 80 100 and 120% of theoretical concentration. The mean recovery of spiked samples obtained was in range of 99.06 to 101.03 reveals no interference of excipients and shows that method is accurate (table5). The proposed validated method was successfully applied to determine aceclofenac in tablet form. The results obtained for tablets of aceclofenac were comparable with the corresponding labeled amounts (0.5 mg/tab) (table3) Robustness of the method was estimated by changing the mobile phase composition (3±3), wavelength ±1 nm, injection volume (20±2µl), column temperature (40±30) and RSD values for all these changes calculated were less than 2 indicate that proposed method is robust.

The proposed RP-HPLC method was accurate, precise, sensitive and rapid. The method also can be extended for the routine analysis of aceclofenac in tablet dosage form.

CONCLUSION

It is thus concluded that the proposed method is new, simple, cost effective, accurate, safe, free from pollution and precise and can be successfully employed in the routine analysis of this drug in pharmaceutical tablet dosage forms. The proposed method shall prove equally effective to analyze aceclofenac in the corresponding drug sample and may prove to be of great importance in pharmaceutical analysis.

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Table 1: HPLC instrumentation & chromatographic conditions for Aceclofenac

Parameters	Description
Instrument	A HPLC instrument (Younglin series) with Model Acme-9000
Column	Promosil C-18, (250 mm, 4.6 mm, 5µm)
Mobile Phase	Different mobile phase used for Trial 1 to 6
Flow Rate	1.0 mL/minute
Detection wavelength	277 nm
Injection Volume	10µL
Run Time	10 minutes

Table 2: System Suitability Test Parameters

Parameters	RP-HPLC method
Retention time, min	6.60±0.132
Tailing factor	1.048±0.274
Asymmetry factor	1.123±0.472
Theoretical plates	5859±0.774
Resolution	2.895±0.431

Table 3: Regression characteristics and Validation Parameters

Sr. No.	Parameter	Value
1.	λmax (nm)	277
2.	Linearity range	1– 10
3.	Correlation coefficient (r2)	0.999
4.	Regression equation	Y=0.99637X+49793
5.	Intercept (a)	.49793
6.	Slope (b)	0.99637
7.	Limit of detection (LOD µg/ml)	0.235
8.	Limit of quantification(LOQ µg/ml)	0.869
9.	Accuracy (%)	99.06-101.03
10.	Repeatability (RSD, %, n=6)	0.195
11.	Precision (RSD %), Interday (n=3)	0.438-1.080%
12.	Intraday (n=30	0.257-0.712

Table 4: Results of analysis of commercial tablets of Aceclofenac

Tablet Formulation	Label claim(mg)	% Label claim Estimated*(Mean ± S.D.)	% Coeff. of Variation	Standard error
I(ACLOFEN)	100	99.435 ± 1.243	1.365	0.514
II(HIFENAC)	150	99.754 ± 1.509	1.523	0.625
III (ACIFON)	200	99.246 ±1.427	1.305	0.613

*Average of six determinations

Table 5: Recovery studies of commercial tablets of Aceclofenac

Tablet Formulation	Label claim(mg)	Drug added(mg)	% Label claim estimated*(Mean ± S.D.)	% Coeff. of variation	Standard error
I(ACLOFEN)	100	50	99.316 ± 1.513	1.496	0.743
II(HIFENAC)	150	70	99.514 ± 1.397	1.432	0.574
III (ACIFON)	200	100	99.288 ±0.863	0.798	0.465

*Average of six determinations

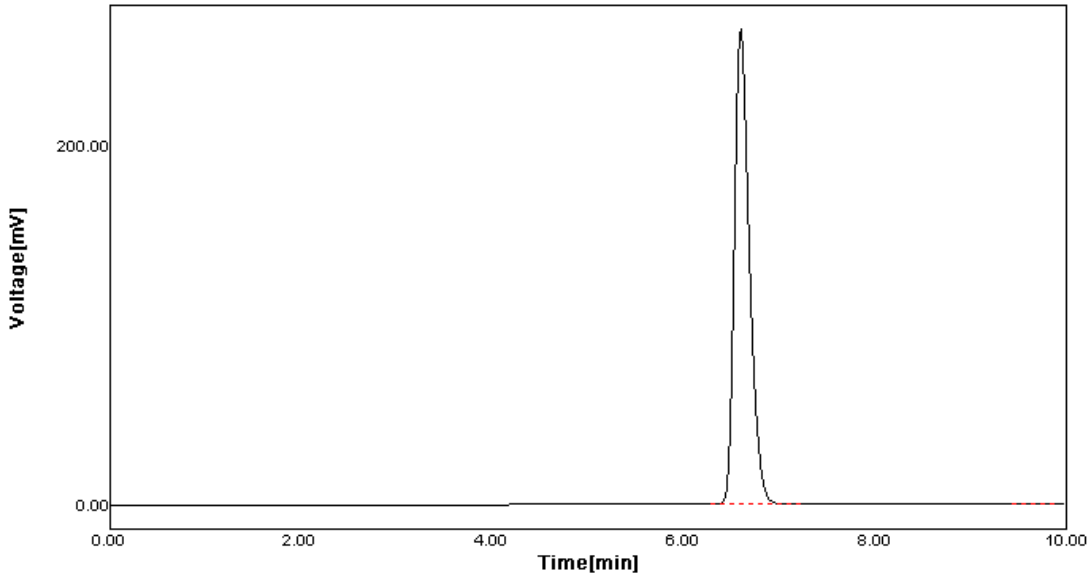

Figure1: Overlay chromatogram of standard Aceclofenac (10 µg/ml)

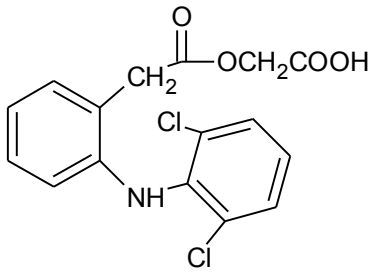

Figure2: Aceclofenac

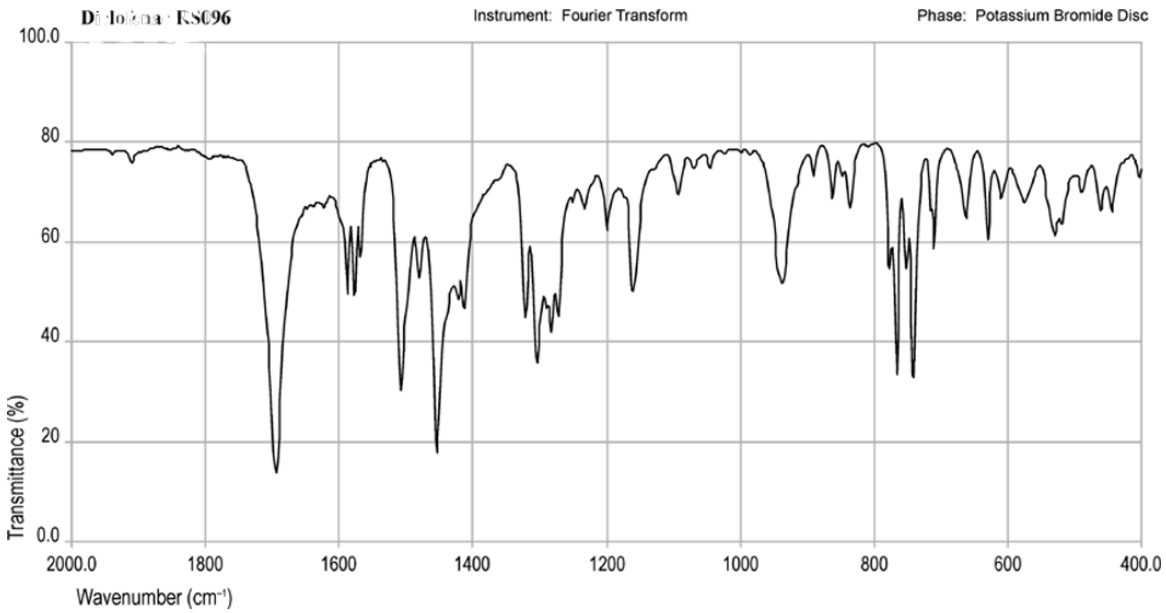

Figure3: Infra Red Spectrum of Aceclofenac

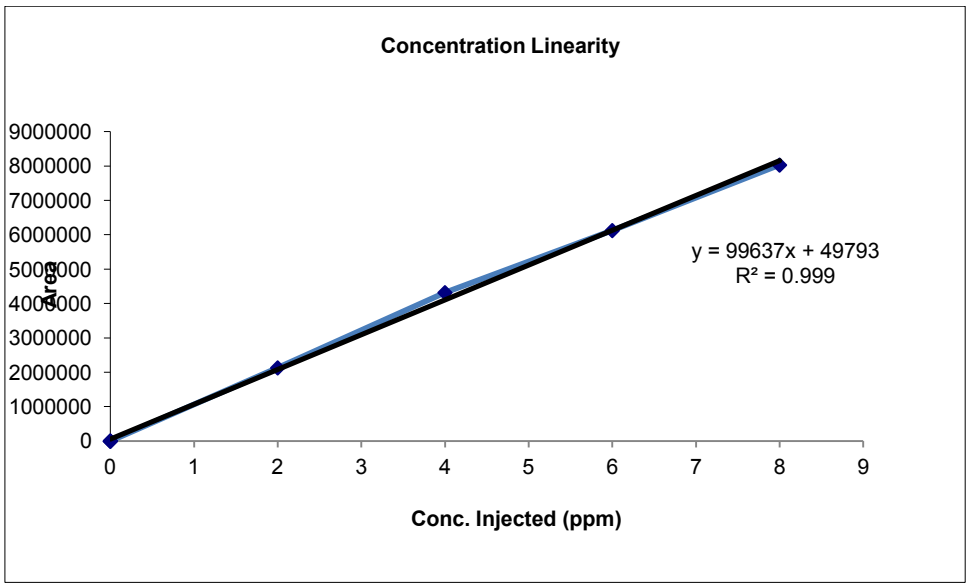

Figure4: Standard Curve of Aceclofenac

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