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Research Article

STANDARDIZATION AN A RP-HPLC METHOD FOR THE ESTIMATION OF ACECLOFENAC IN DOSAGE FORM

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ABSTRACT

A rapid and sensitivity RP-HPLC method was proposed for the qualitative and quantitative estimation of Aceclofenac in dosage form. Aceclofenac had been chromatographed on a C_{18} column with a mobile phase buffer pH 5.0 and Acetonitrile in the ratio of 60:40v/v. The mobile phase was pumped at a flow rate 1ml/min. Etoricoxib was used as internal standard and elutents were monitored at 275nm. The retention time of the drug was 7-10 min. With this method linearity was observed between area under curve and concentration of Aceclofenac in the injected solution, in the range of 25-125µg/ml. The method was found to be applicable for the estimation of Aceclofenac in the dosage form. The results of analysis were validated statistically and recovery studies confirmed the accuracy of the proposed method.

Keywords: RP-HPLC, Aceclofenac, Etoricoxib and C_{18} column.

INTRODUCTION

Analytical chemistry involves separating, identifying and determining the relative amounts of the components in a sample of **matter** [1-5]. Qualitative analysis reveals the chemical identity of the species in the sample. Quantitative analysis establishes the relative amount of one or more of these species or analytes in numerical terms. Aceclofenac is chemically designed as 2-[(2, 6-Dichloro phenyl) amino] benzene acetic acid carboxy methyl. A white /almost white crystalline powder .Practically insoluble in water, freely soluble in acetone and soluble in Alcohol. Aceclofenac is an orally administered NSAID which is a phenyl acetic acid derivative. It inhibits the prostaglandin synthesis. It is a potent inhibitor of the enzyme Cox which is involved in the synthesis of prostaglandins. It is used in the management of Osteo arthritis, Rheumatoid arthritis and Ankylosing spondylitis. The usual dose of Aceclofenac is 100mg twice a day. In patient with hepatic impairment an initial daily dose of 100mg can be used. Aceclofenac tablet should be swallowed whole with a sufficient quantity of liquid.[6-9]

Literature survey reveals few methods have been reported for the Aceclofenac in human plasma by RP-HPLC[10]. Fast-free zone electrophoresis method for the simultaneous determination of Aceclofenac and Diclofenac in human plasma were also reported11. The aim of the present study was to develop a simple, precise, rapid, and accurate RP-HPLC method for single dosage form.

MATERIALS AND METHODS

Materials

Drug sample

Aceclofenac was obtained as gift sample from Ipca Laboratories Ltd., Mumbai and Etoricoxib obtained from Cadila Pharma Ltd. Ahmadabad, for present study.

Chemical and Solvent used

Acetonitrile HPLC grade, Disodium hydrogen orthophosphate AR grade, Orothophosphoric acid AR grade, was supplied by Qualigens fine chemicals Ltd., India. Water of HPLC grade was obtained from Milli-Q RO system.

Instruments Used

A. Sartorius Digital Balance (R2000 and 1720)
B. Systronics- pH meter, upH system 361.
C. Shimadzu UV 160A recording spectrophotometer.
D. Shimadzu HPLC with the following configurations:
1. Shimadzu LC-10Atvp solvent delivery system
2. Rheodyne 7725i with 20ul loop
3. SPD-M 10Avp photo diode array detector
4. Class VP data system.

Analytical column

(a) Shimpak, C_{18}, 5u, 25cm X 4.6mm i.d
(b) Phenomenex Gemini C_{18},5u, 25cm X 4.6mm i.d
(c) Lichrocart Purospher C_{18},5u, 25cm X 4.6mm i.d

Estimation of Aceclofenac dosage form by HPLC method

Estimation of Aceclofenac in dosage forms by HPLC method was carried out. This section describes the chromatographic condition, selection of wave length, the preparation of mixed standard and sample solutions, procedure for recording and integrating the chromatograms.

Each tablet contains
ZERODOL -100mg (Aceclofenac)
Manufactured by –Ipca laboratory, Mumbai.

Selection of wavelength

An injection of 100µg/ml of each of the standard solution of Aceclofenac was made and the PDA profile was recorded (200-400nm). From the PDF profile, a detection wave length of 275nm was selected, as both the drugs gave better peak response.

Preparation of standard solution

- 100mg of Aceclofenac was taken into 100ml standard flask to this 25ml mixture of Acetonitrile: water (1:1v/v) was added and vortexed for about 10min (until the clear solution was obtained) and made up to 100ml with mobile phase. 5ml of this solution was further diluted to 50ml with mobile phase (standard stock solution A).
- 10ml of Standard stock A solution was taken in 50ml of standard flask and made up to 50ml with mobile phase(standard solution A)
- 50mg of Etoricoxib was taken into 50ml of standard flask to this 30ml of mixture of Acetonitrile: water (1:1v/v) was added, sonicated for 10min until clear solution was obtained and made up to 50ml with mobile phase(standard stock solution B)
- 20ml of standard stock solution B was taken into 50ml of standard flask and made up to 50ml with mobile phase (standard solution B)
- 10ml of standard solution A and 10 ml of standard solution B was diluted to 50ml with mobile phase (standard solution C , which consist 100µg/ml of Aceclofenac and 200 µg/ml of Etoricoxib) used for analysis.

Preparation of sample solution

- 20 tablets, each containing 100mg of Aceclofenac were weighed and finely powdered, from the powdered tablet, a quantity of powder equivalent to 100mg of Aceclofenac was taken into a sintered glass crucible to this 200mg of Etoricoxib was added and then extracted with, each of, 25ml of the mixture of Acetonitrile and water (1:1 v/v) for three times. The combined extracts were made up to 100ml with mobile phase and filtered. (sample solution A)
- 10ml of sample solution A was diluted to 100ml with mobile phase and used for estimation. This solution contains 100µg/ml of Aceclofenac and 200 µg/ml of Etoricoxib.(sample solution B)

Chromatographic conditions

Stationary phase: Phenomenex Gemini C_{18}, 5u, 25cm X 4.6mm i.d
Mobile phase: Solvent A: Phosphate buffer (pH 5.0)
Solvent B: Acetonitrile
Solvent ratio: 60:40 (v/v A and B)
Detection of wave length: 275nm

Flow rate: 1.0ml / min
Temperature: Room temperature about 20°c
Internal standard: Etoricoxib

Method of Analysis

With optimized chromatographic condition baseline was recorded after the stabilization of the baseline for about 20min. Successive 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 injections of sample test solution were bracketed by injection of standard solution. The procedure was repeated for six times and response factor of standard peak and sample peak were calculated.

Validation of the method

Accuracy of the method was determined by recovery experiments. To the formulation, reference standard of respective drugs were added at the level of 25% and 50% of the label claim. Precision of the developed method was carried out by determining interday and intraday variation studies. The response factor and %RSD of the response factors were calculated. The LOD and LOQ of developed method were determined by analyzing progressively lower concentration of the standard solution using the optimized chromatographic conditions.

System stability studies were carried out as specified in the United States Pharmacopoeia (USP). These parameters include column efficiency, resolution, peak asymmetry factor, and capacity factor and percentage coefficient of variation of peak area or height on repetitive injections. Although the USP requires only two of these criteria for method validation, parameters like column efficiency (N), resolution (R_s) and peak asymmetry factor were calculated in the present study. Chromatograms of mixed standard solutions were used for these calculations.

RESULTS AND DISCUSSIONS

The sensitivity of HPLC method that uses UV detection depends upon the proper selection of wavelength. Mixed standard solutions were injected and the PDA profiles were recorded (200-400nm). The overlapping spectra and PDA chromatogram are shown in fig 3-4. From the PDA profile the detection wavelength of 275nm was selected which gave better peak response.

Estimation of Aceclofenac in dosage forms by HPLC method was carried out under optimized chromatographic condition. The standard solution and sample solutions were prepared. The chromatograms were recorded. The typical chromatograms of the mixed standard and sample solutions are given in fig.1 and 2 respectively.

The response factor of standard and sample solutions was calculated. The assay procedure was repeated for six times. The percentage of individual drugs found in formulations, standard deviations were calculated and presented in Table 1. The result of analysis shows that the amount of drug was in good agreement with the label claim of the formulations

The accuracy of the method was determined by the recovery experiments. The recovery studies were carried out six times and the % recovery; standard deviations were calculated and presented in Table 2. From the data obtained, added recoveries for the standard drugs were accurate.

The precision of the method was demonstrated by inter and intraday variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of the drug peaks and percentage RSD were calculated and presented in Table.3. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factors of drug peaks and percentage RSD were calculated and presented in Table 3.

The standard drug solution of varying concentration ranging from 50% to 150% of the assay of targeted level of assay concentration 25.0µg/ml to 125.0µg/ml of Aceclofenac containing 200µg/ml of Etoricoxib were examined by the assay procedure. The calibration curve were plotted using response factor versus concentration of standard solutions were given in fig.5. The calibration graph shows that the linear response was obtained over the range of concentration used in assay procedure. The calibration curves of combination pass close to the origin, which justifies the use of single point calibration and the values are given in Table 4. These data demonstrates that the method have adequate sensitivity to the concentration of the analytes.

The resolution, capacity factor, theoretical plates/meter, peak symmetry and Rf value were calculated for the standard solutions and presented in Table 5. The values obtained demonstrated the suitability of the system for the analysis of the above drug combinations. System suitability parameters might be fall within ±3% standard deviation range during routine performance of the method.

CONCLUSION

In the developed HPLC method, the sample and standard preparation requires less time, no tedious extraction procedures were involved; run time required for recording the chromatograms was less. This demonstrates that developed HPLC method is simple and rapid. The proposed HPLC method for the estimation of Aceclofenac in dosage form is accurate, simple and rapid. Hence the present HPLC method is suitable for the quality control of the raw materials, Formulations and dissolution studies.

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Table 1: Analysis of formulation

DRUG	Labeled amount (mg/tablet)	Amount taken for Assay (µg/ml)	HPLC Determination	
			Amount found* (µg/ml)	%Label claim*
Aceclofenac	100.0	100.0	98.12±0.7894	98.12±1.241

*Average of 6 determination

Table 2: Recovery studies

Level	Aceclofenac		
	Concentration of drug added (µg/ml)	Amount of drug recovered (µg/ml)	Recovery (%)
Level 1	50.0	48.99	Mean :97.98 CV : 2.31 N :6
Level 2	100.0	98.29	Mean :98.29 CV :2.54 N :6
Level 3	150.0	148.41	Mean :98.94 CV :2.63 N :6

Table 3: Precision Studies

Intra day studies	Day	Inter day studies
Mean concentration of Aceclofenac* (100µg/ml)		Mean concentration of Aceclofenac (100µg/ml)
98.56±0.514	Day 1	98.96 ± 0.287
	Day 2	98.78 ± 0.314
	Day 3	98.51 ± 0.365

*Average of 6 determination

Table 4: Linearity and Range

CONCENTRATION OF ACECLOFENAC (µg/ml)	CONCENTRATION OF INTERNAL STANDARD (µg/ml)	Response Factor
25.0	200.0	0.157
50.0	200.0	0.313
75.0	200.0	0.472
100.0	200.0	0.635
125.0	200.0	0.788

Response factor=Peak Area of Standard/peak Area of Internal standard

Table 5: System Suitability Studies

S.No	PARAMETERS	ACECLOFENAC	INTERNAL STANDARD
1	Theoretical plate/meter	25417	21514
2	Resolution factor	1.25	
3	Asymmetric factor	0.91	1.01
4	LOD(ng/ml)	5	10
5	LOD(ng/ml)	25	50

Fig 1. Typical chromatogram of standard solution of Aceclofenac

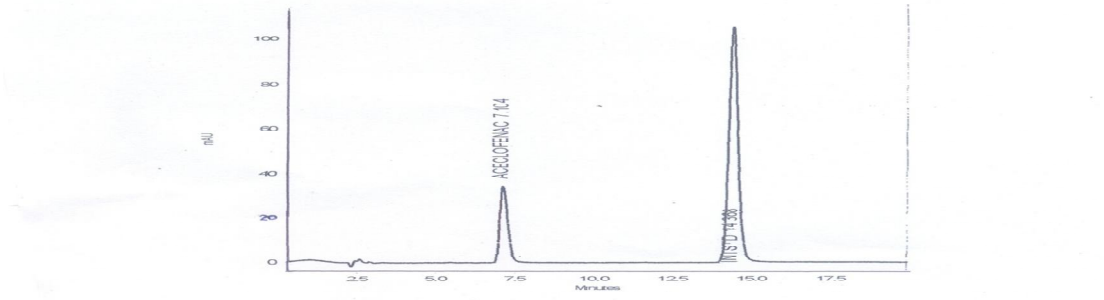


Fig 2. Typical chromatogram of sample solution of Aceclofenac

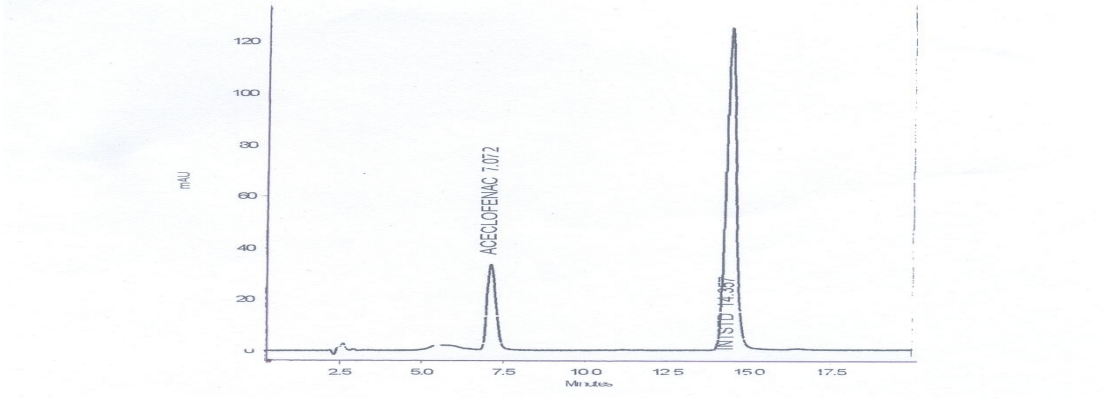


Fig 3. Overlaid UV spectrum of Aceclofenac and Internal standard

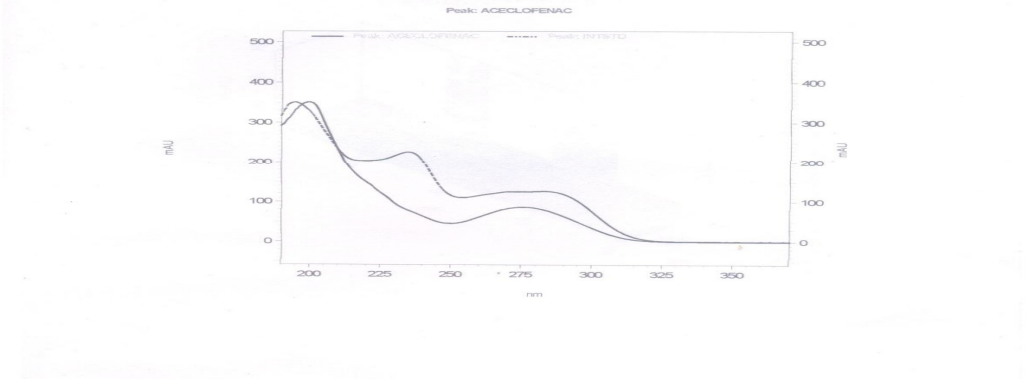


Fig 4. PDA Chromatogram of Aceclofenac

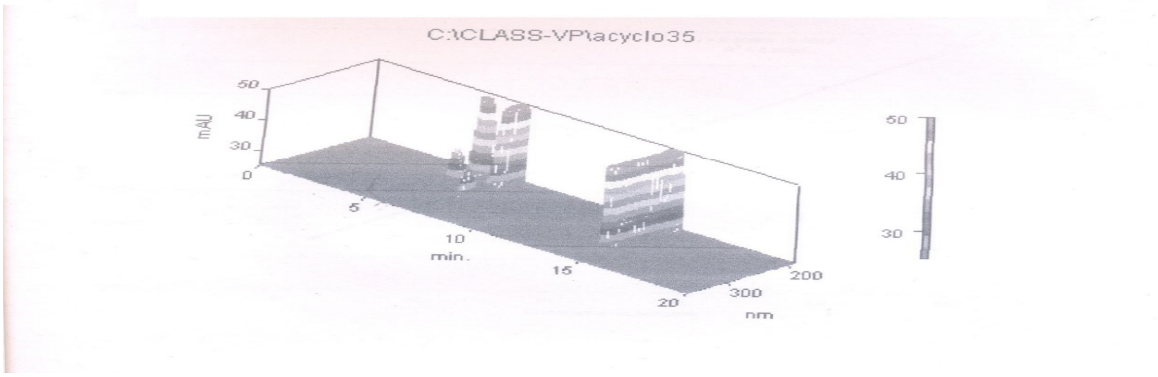
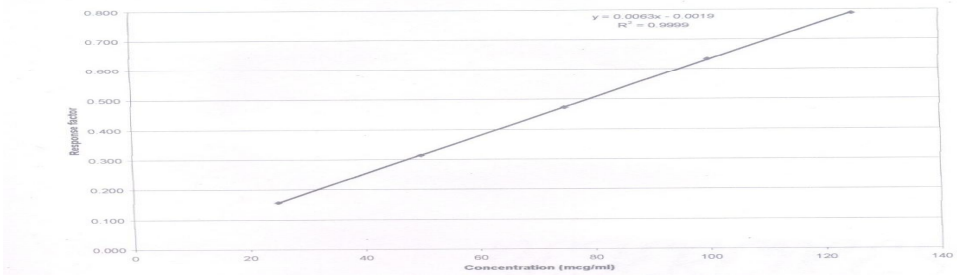


Fig 5. Calibration curve of Aceclofenac



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