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Research Article

A VALIDATED REVERSED PHASE HPLC-METHOD FOR THE DETERMINATION OF ACECLOFENAC AND TIZANIDINE IN TABLETS

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ABSTRACT

A new simple, accurate, precise and reproducible Reverse Phase-High Performance Liquid Chromatographic method has been developed for the simultaneous estimation of Aceclofenac and Tizanidine in tablet dosage forms using C_{18} column (Ineretsil, 250 x 4.6 mm, 5 μm) in isocratic mode. The mobile phase consisted of acetonitrile, methanol and 20 mM phosphate buffer adjusted to pH 3.5 in ratio of (40:30:30 v/v) with Ultraviolet-Visible detection at 230 nm. The method was linear over the concentration range for aceclofenac120-280 μg/ml and for tizanidine 2-40 μg/ml. The recoveries of Tizanidine and Aceclofenac were found to be in the range of 99.45-100.61% and 99.56-101.32% respectively. The validation of method was carried out using International Conference on Harmonization-guidelines. The described High Performance Liquid Chromatographic method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form.

Key words: Simultaneous estimation, RP-HPLC, Aceclofenac, Tizanidine, ICH guideline

INTRODUCTION

Aceclofenac, {2, (2,6dichlorophenyl) amino phenyl acetic acid.}, is a phenyl acetic acid derivative (Structure 1a) with the improved gastric tolerance and is used for relief pain and inflammation in rheumatoid arthritis[1]. Tizanidine is chemically {5, chloro N- (2 imidozolin 2yl) 2, 1, 3, benzothiadiazol 4- yl amine hydrochloride} (Structure 1b), is centrally acting skeletal muscle relaxant with central analgesic effect and gastric tolerance effect with combination on Non-Steroidal Anti-Inflammatory Drugs[2]. A combination of these drugs containing Tizanidine and Aceclofenac is commercially available by Aceclofenac 100 mg and Tizanidine 2 mg for rheumatic disorders. Aceclofenac and Tizanidine individually have shown efficacy in the treatment of low back pain. But Aceclofenac-Tizanidine combination was more effective than aceclofenac alone and had a favorable safety profile in the treatment of acute low back pain[3].

Literature survey revealed that various analytical methods like spectrophotometric, HPLC and High Performance Thin Layer Chromatographic methods have been reported for the determination of Aceclofenac and Tizanidine from their formulations individually and in combination with other drugs[4-16]. The review of literature revealed that no method is yet reported for the simultaneous estimation of both the drugs in combination. This prompted us to develop simple, accurate, precise and sensitive simultaneous estimation of Aceclofenac and Tizanidine by RP-HPLC method. The method was validated as per International Conference on Harmonization (ICH) guidelines[17].

EXPERIMENTAL PROCEDURE

Drugs and chemicals:

The pharmaceutical grade pure samples of Aceclofenac (99.26%) and Tizanidine (99.64%) supplied by Healthcare Pharmaceuticals, Pondicherry, India. Acetonitrile and methanol HPLC grade solvents; all analytical grade solvents obtained from E-Merck Ltd, Mumbai, India. Potassium dihydrogen orthophosphate Analytical Reagent grade was procured from Qualigens Fine Chemical, Mumbai, India. The HPLC grade water was obtained from a Milli-Q water purification system.

HPLC apparatus and conditions:

The separation was performed by using Ineretsil C_{18} (250 × 4.6 mm, 5 μm) column on a Shimadzu liquid chromatographic system equipped with a Shimadzu LC 10 AT VP isocratic solvent delivery system, Shimadzu SPD 10A dual wavelength absorbance detector and Rheodyne injector with 20 μl loop volume. Mobile Phase consisted of 20 mM phosphate buffer (adjusted to pH 4): acetonitrile : methanol in ratio of (40:30:30 v/v). The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 1.0 ml/min. and the detector wavelength was set at 230 nm. The injection volume was 20 μl (fixed loop).

Stock solutions and standards:

Standard stock solutions were prepared of 1000 μg/ml of Aceclofenac and Tizanidine, separately using mobile phase. From the standard stock solution different concentrations of working standard solution were prepared ranging from 120-280 μg/ml for Aceclofenac and 2-40 μg/ml for Tizanidine.

Calibration curve:

The calibration curves were constructed for the determination of the linearity and the curves were plotted with the concentration range verses area must obey Beer's law. The linearity was evaluated by analysis of the serially diluted sample in the range of 2-40 μg/ml for Tizanidine and 120-280 μg/ml for Aceclofenac. An aliquot was injected using mixture of 20 mM phosphate buffer: acetonitrile: methanol (40:30:30 v/v). The 20 μl mixture was injected for the estimation under the optimized chromatographic conditions. The typical chromatogram was recorded for standard as shown in Fig 1. The retention time of standard Tizanidine and Aceclofenac were found to be 4.32 min. and 6.93 min. respectively with a good resolution of 14.63.

Analysis of formulations:

Twenty tablets were weighed and finely powdered. A quantity equivalent to 20 mg of Tizanidine and 100 mg of Aceclofenac were transferred to 100 ml volumetric flask and dissolved on about 50 ml of mobile phase. The solution was ultrasonicated for 10 min and filtered through Whatmann filter paper No.41 and the final filtration was done in 0.45 micron membrane and volume made up to mark with same solvent system. Above solution was taken to prepare a dilution of 4 μg/ml of Tizanidine and 200 μg/ml of Aceclofenac. The amount of drug was determined and three replicate injections were done (Table-3).

RESULT AND DISCUSSION
Method development:

Several tests were performed in order to get satisfactory separation-resolution of Aceclofenac and Tizanidine in different mobile phases with various ratios of organic phase and buffers by using C_{18} column. The ideal buffer was used 20mM phosphate buffer (pH 4): acetonitrile: methanol in ratio (40:30:30 v/v) by isocratic elution to obtain satisfactory and good resolution. Increasing or decreasing pH of mobile phase by ± 0.2 dose not shows significant change in retention time of each analyte. The retention of Aceclofenac and Tizanidine on analytical column was evaluated at a flow rate of 1.0 ml/min. and the injection volume was 20 μl. The retention time of standard and sample for Aceclofenac and Tizanidine were satisfactory with good resolution.

Linearity:

The linearity for HPLC method was determined at six concentration levels. The linearity of Aceclofenac and Tizanidine were determined by calibration curves and the linearity based on the area observed in the range of 2-40 μg/ml for Tizanidine and 120-280 μg/ml for Aceclofenac. The % Relative standard deviation (% RSD) of peak area and the retention time was within the limit of ±2%. This indicates that the method was system suitable. The reports are tabulated below in Table 1. The regression co-efficient value (r^2) for Aceclofenac and Tizanidine is 0.9996 and 0.9990 respectively.

Precision:

Precision was measured for both inter and intra-day, and checked with repeatability and the % RSD for the repeatability was found to be 0.171% to 0.254% and 0.134% to 0.283% respectively for Aceclofenac and Tizanidine. The RSD was found to within the limit and tabulated in Table 1. The limit of quantification was determined by injecting minimum concentration of the drugs. The limit of quantification was found to be 50 μg /ml and 0.80 μg/ml for Aceclofenac and Tizanidine.

Recovery studies:

The assay procedure was repeated for standard and sample six times and mean peak area ratio and concentration of drugs were calculated. The percentage of individual drugs found in formulation, mean and % RSD in formulation were calculated and present in Table 2. Recovery study carried out for both the drugs was performed by spiking the known amount of pure drug in powdered formulations. It is usually done by adding 80 %, 100 % and 120 % of the pure drug with the formulation taken for analysis. The average % recovery for Aceclofenac and Tizanidine was found to be 99.57 % to 99.62 % respectively. The results are tabulated below in Table 3.

Specificity and Selectivity:

Specificity was tested against standard compounds and against potential interferences. To determine specificity with respect to sample compounds the responses of standard and sample solution were compared. No interferences were detected at the retention times of either Aceclofenac or Tizanidine in sample solution.

The limit of detection (LOD) was determined as lowest concentration giving response and limit of quantification was determined as the lowest concentration analyzed with accuracy method were determined by injecting progressively low concentrations of the standard solutions using developed RP-HPLC method. The limit of detection (LOD) for Aceclofenac and Tizanidine was found to be 20 μg /ml and 0.40 μg/ml respectively. The limit of quantification (LOQ) was 50 μg /ml and 0.80μg/ml for Aceclofenac and Tizanidine respectively and reported Table 1.

Stability:

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. The results show that for both solutions, the retention time and peak area of Aceclofenac and Tizanidine remained almost similar (% RSD less than 2.0) and significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 h, which was sufficient to complete the whole analytical process.

Table- 1

SYSTEM SUITABILITY PARAMETER

| PARAMETERS	Aceclofenac	Tizanidine
Calibration Range (μg /ml)	120-280	2-40
Correlation Coefficient(r2)	0.9999	0.9998
Retention time(Min)	6.9±0.2	4.9±0.2
Asymmetry	1.127	1.148
Theoretical Plates	4563	3269
Resolution factor	14.63	---
Tailing Factor	1.36	1.12
Selectivity	--	1.60
Repeatability %RSD (n=5)	0.246%	0.283%
Limit of quantification (μg /ml)	50	0.80

Table- 2

ANALYSIS OF MARKETED FORMULATION

Formulation	Aceclofenac			Tizanidine		
	Label claim mg/tab	Amount found* mg/tab ± RSD	% assay ± RSD	Label claim mg/tab	Amount found* mg/tab ± RSD	% assay ± RSD
ASMR	100	101.36±0.369	101.36±0.963	2	1.96±0.958	99.95±0.654

* stands for the average reading taken in three readings.

Table- 3

RECOVERY STUDIES OF TIZANIDINE AND ACECLOFENAC IN COMBINED DOSAGE FORM

Formulation	Aceclofenac			Tizanidine		
	% added	% recovered* ± RSD	% recovery ± RSD	% added	% recovered* ± RSD	% recovery ± RSD
Brand ASMR	80	79.89±0.423	99.56±0.015	80	79.58±0.589	99.45±0.380
	100	99.82±0.892	99.82±0.010	100	100.61±0.632	100.61±0.010
	120	121.21±1.126	101.32±0.065	120	120.58±0.654	100.38±0.221

*Recovery experiment data for Aceclofenac and Tizanidine showing the amount of drug recovered from sample solution at each level (n=3), percentage recovery and the average percentage recovery.
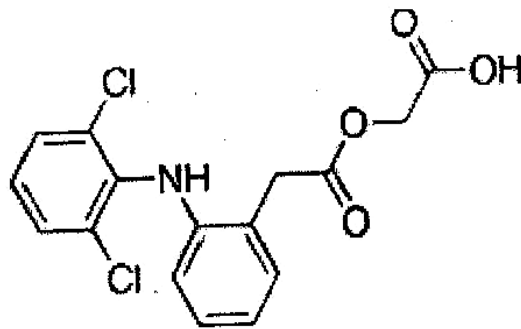
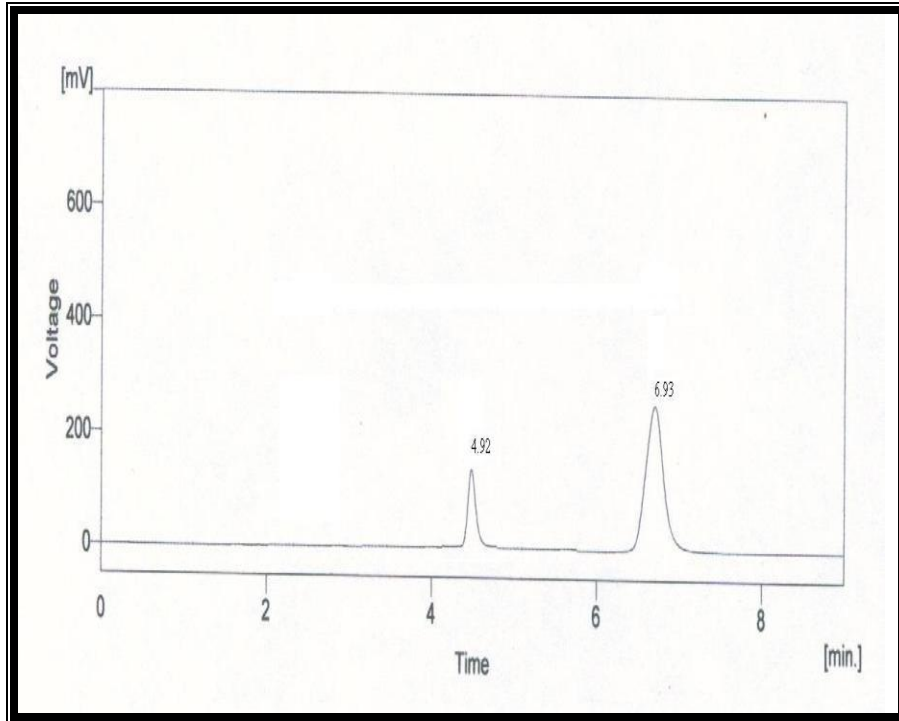1 (a): Structure of Aceclofenac
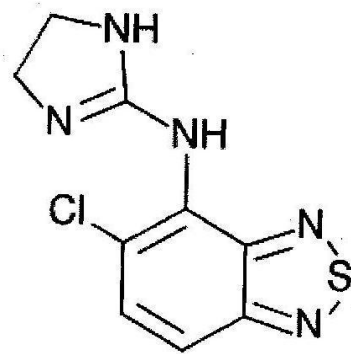
1 (b): Structure of Tizanidine

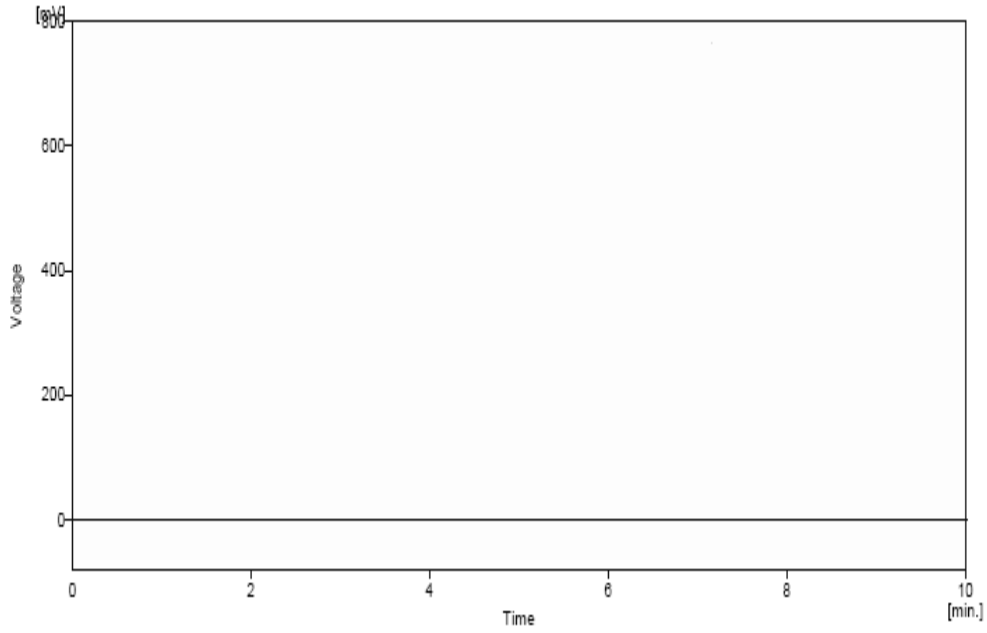Fig 1: A Typical Chromatogram for Aceclofenac and Tizanidine
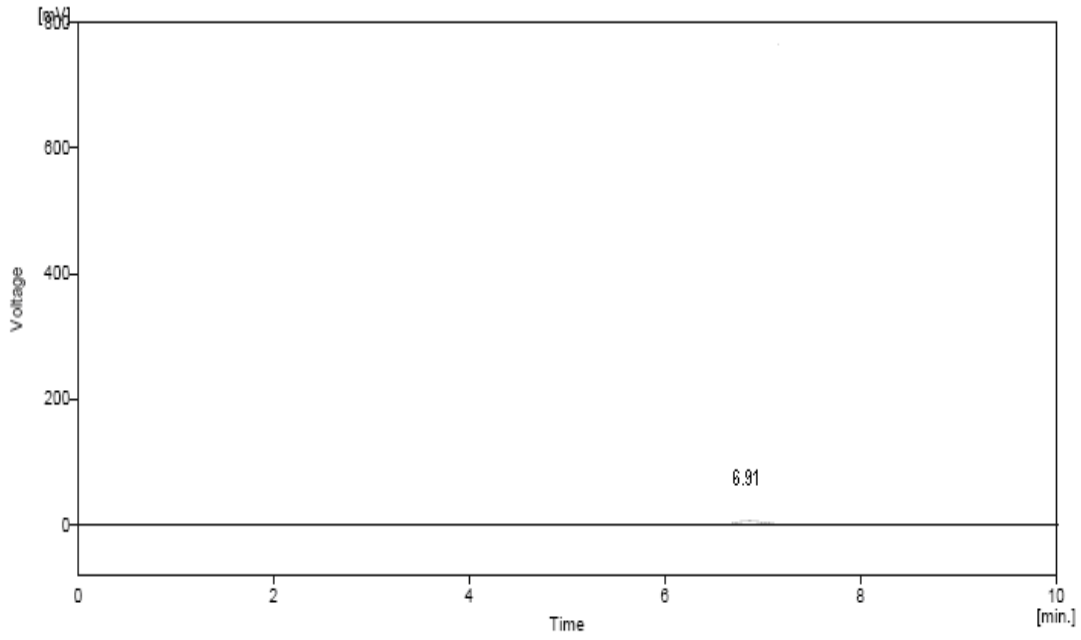

Fig 2 (a) Blank

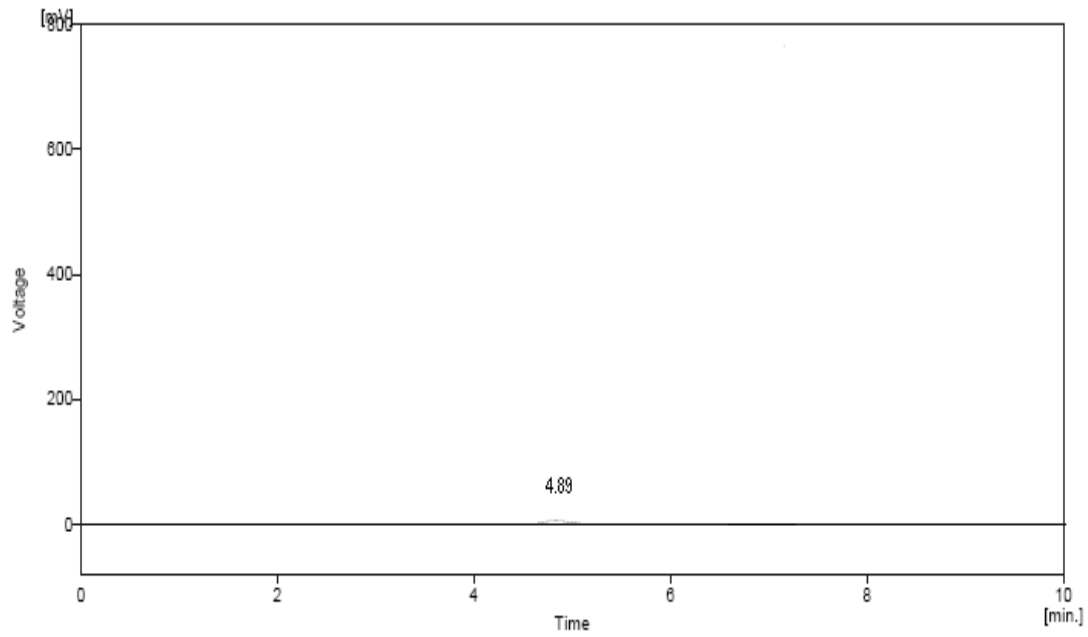Fig 2 (b) Limit of Detection (LOD) of Aceclofenac 20μg/ml

Fig 2 (c) Limit of Detection (LOD) of Tizanidine 0.40μg/ml

Ruggedness and Robustness:

Ruggedness test was determined between two analysts, instruments and columns. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

CONCLUSION

A selective, sensitive, precise and accurate method has been developed for the analysis of Aceclofenac and Tizanidine in Tablet dosage form. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

ACKNOWLEDGEMENTS

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