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Method Development And Validation For Simultaneous Determination Of Aceclofenac And Tizanidine In Bulk And Marketed Formulation

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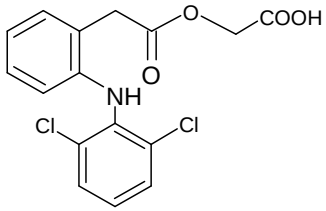
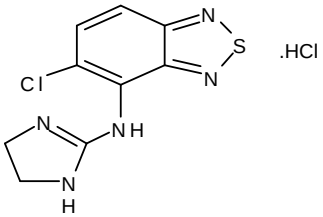
Abstract: A reverse phase high performance liquid chromatographic method (HPLC) has been developed for the simultaneous estimation of Aceclofenac (ACE) And Tizanidine (TZN) in the pharmaceutical formulation And Bulk Drug using C18 column. The mobile phase (Acetonitrile and Phosphate Buffer pH 3.5) was pumped at a flow rate of 1 ml/min in the ratio of 60:40 and the eluents were monitored at 293 nm. Linearity was obtained in the concentration range of 20-120 µg/ml for Aceclofenac & 5-30 µg/ml. The method was statistically validated and RSD was found to be less than 2% indicating high degree of accuracy and precision of the proposed HPLC method. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining Aceclofenac And Tizanidine in bulk drug samples or in pharmaceutical dosage form.
Key Words: Aceclofenac,Tizanidine, High Performance Liquid Chromatography.

Introduction[1-8]

Aceclofenac (ACE) is an anti-inflammatory class of drug And official in European Pharmacopoeia. Tizanidine is Skeletal muscle relaxant and official in IP-2007, USP 30-NF 25. Aceclofenac and Tizanidine are available in combined tablet dosage form as an analgesic and Muscle Relaxant. Aceclofenac relieves pain by stimulating cartilage synthesis, Tizanidine is a short acting drug for the management of spasticity. It is an agonist at a 2-adrenergic receptor site & reduces the spasticity by increasing presynaptic inhibition of motor neurons. Literature survey reveals that few UV, HPLC, HPTLC and colorimetric methods have been reported for the estimation of Aceclofenac & Tizanidine as single component formulation and combination with other drugs in bulk samples, formulations However not a single HPLC method is reported for the simultaneous analysis of the two drugs in bulk and combined dosage form. Fixed dose combination Aceclofenac (100mg), Tizanidine(2mg) are available in tablet form in the market. The present study describes the determination of Aceclofenac,Tizanidine in bulk drug sample and pharmaceutical dosage form using Thermo BDS hypersil C18 (250 mm × 4.6 mm, 5 µm) with UV detection at 293.0nm. The method was validated according to procedures and acceptances criteria based on FDA & ICH guidelines.



Aceclofenac



Tizanidine

Experimental[1-8]

Material And Reagents:

1. Pure drugs of Aceclofenac was obtained as gift samples from Inventia Health Care., Mumbai, India, And Tizanidine were gifted by Lupin Ltd. Pune.
2. Acetonitrile,Methanol and Water were of HPLC grade.
3. All other reagents used in the study were of AR grade
4. The Acent –tz Tablet(Label to contain 100mg of Aceclofenac and 2mg of Tizanidine is obtain from Intra Labs,India.
5. Phosphate Buffer pH 3.5(dissolved 3.4 gm of Sodium Dihydrogen Phosphate in water and made upto 1000 ml with water. The pH is adjusted with 1%Tri-ethylamine and O- phosphoric acid)
6. filters used 0.45µ Nylon syringe membrane filters
7. The mobile phase consisting of Acetonitrile : 25 mM Phosphate buffer pH 3.5 (60:40, v/v) adjusted with triethylamine and O-phosphoric Acid, filtered before use through 0.2 µm nylon filter paper.

Instrumentation:

The HPLC system (Make: Shimadzu, Model: LC-2010 CHT) consisted of a pump with auto injection facility. The capacity of loop was 20 µl. The detector consisted of a UV-VIS spectrophotometer operated at a wavelength 293 nm. The software used was LC solution version 1.25. The column used was Thermo BDS hypersil C18 (250 mm × 4.6 mm, 5 µm). Absorbance measurements were made on double beam UV-Visible spectrophotometer (Make: Shimadzu, Model: UV-1800) with 1 mm quartz cell.. The digital pH meter used was of (Make: Equip-Tronics, Model: EQ-610, India). All the weighings were carried out on electronic balance (Make:Shimadzu, Model: AX 200, Japan) and sonication of mobile phase was done using ultrasonic cleaner (Make: Spectra lab, Model: UCB-40, Mumbai).

Chromatographic Conditions

Separation was achieved using a Thermo BDS hypersil C18 (250 mm × 4.6 mm, 5 µm) Column. mobile phase pumped at a flow-rate of 1 ml/min consisted of Acetonitrile : 25 mM Phosphate buffer pH 3.5 (60:40, v/v) adjusted with triethylamine and O-phosphoric Acid prepared daily and degassed by passing through a 0.2 µm membrane filter and ultrasonication for 10 min. All separations were performed at temperature 35^0C with detection at 293nm.

Standard Solution

A standard solution of Aceclofenac(100mg) and Tizanidine(2mg) were prepared in methanol. Subsequent dilutions were made in mobile phase to give the concentrations 20, 40, 60, 80,100 and 120 µg/ml for Aceclofenac And 5, 10, 15, 20, 25 and 30 µg/ml for Tizanidine.

Assay Of Tablet Formulation:

To determine the content of drugs in tablet formulation acent-TZ label claim: 100 mg aceclofenac and 2 mg tizanidine per table, the twenty tablets were weighed, their mean weight determined and they were finely powdered and powder equivalent to 100 mg aceclofenac 2 mg tizanidine was weighed. Then, equivalent weight of the drug was transferred into a 100 ml volumetric flask containing 50 ml mobile phase, sonicated for 30 min and diluted to 100ml with mobile phase. The resulting solution was then filtered using 0.45µ filter.

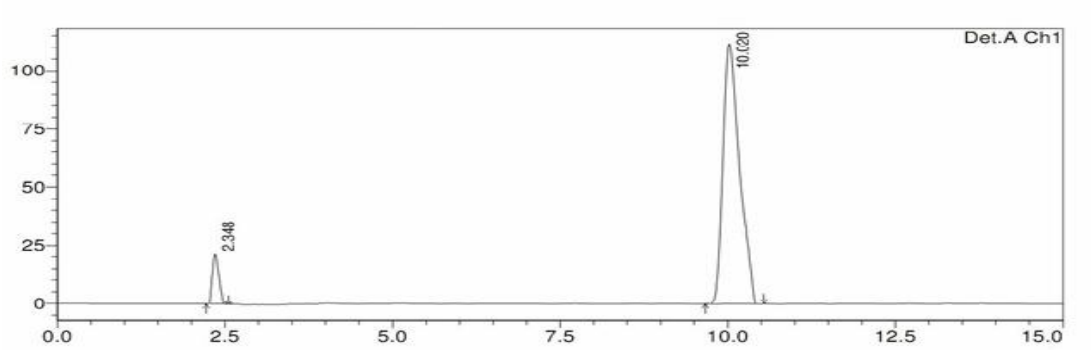


Figure 1. Standard Chromatogram of Aceclofenac and Tizanidine

Table 1: Validation And System Sutiability Studies

Parameter	Aceclofenac	Tizanidine
Linearity range (µg/ml)	20-120	5-30
Intercept	26667	5933
Slope	21228	8181
Correlation coefficient	0.998	0.997
(%) R.S.D	0.05	0.05
Limit of detection (µg/ml)	0.03	0.01
Limit of quantization (µg/ml)	0.01	0.04
Retention time (min.)	10.02	2.34
Robustness	Robust	Robust
Precision (% R.S.D)		
Inter-day (n=3)	0.11	0.02
Intra day (n=3)	0.12	0.11
Tailing factor	1.2	0.97
Theoretical Plates	7902.56	2023.70
Mean % recovery	99.70	99.69
Resolution factor	Between TZN and ACE is 7.68	

Table 2: Analysis Of Tablet Formulation

Component	Amount Present (mg)	Amount found *(%)	Standard Deviation*	% RSD*
ACE	100	99.69	0.378	0.378
TZN	2	99.70	0.615	0.615

*Denotes average of six determinations.

ACE,TZN for Aceclofenac and Tizanidine Respectively.

Table 3: Recovery Studies And Its Statistical Validation Data

Level of % Recovery	Component	Amount present (mg)	Amount of standard added(mg)	% Recovery*	Standard deviation*	% RSD*
80	ACE	100	50	100.02	0.005	0.011
	TZN	2	50	100.15	0.372	0.748
100	ACE	100	100	99.70	0.615	0.615
	TZN	2	100	99.69	0.378	0.378
120	ACE	100	150	99.99	0.450	0.301
	TZN	2	150	100.09	0.099	0.066

* Denotes average of three determinations at each level of recovery.

Results And Discussion

Chromatographic Conditions

Optimization of chromatographic conditions was achieved by monitoring varying mobile systems. After trying different ratios of mixtures acetonitrile:Phosphate Buffer pH3,Methanol:Water,The best results were achieved by using a mixture of Acetonitrile : 25 mM Phosphate buffer pH 3.5 (60:40, v/v) as mobile phase. Excellent chromatographic specificity, Moreover, suitable retention time for Aceclofenac and Tizanidine were achieved. Typical chromatograms obtained from the standard solution of Aceclofenac and Tizanidine is presented in **Fig.1.** Under the chromatographic conditions described,Aceclofenac and Tizanidine were well resolved and eluted at about 2.03 and 10.02 min Respectively. The total run time was within 15 min. Good baseline resolution and peak shape can be observed. Also, the influences of small changes in the mobile phase composition (±10%) was studied to determine the robustness of the method, such as the changes in peak area and retention time.

Linearity

Calibration curves were constructed using five series of standard solutions of Aceclofenac and Tizanidine. aceclofenac in the range of 20-120 µg/ml, and Tizanidine as 5-30 µg/ml. A linear relationship was obtained between A linear relationship was obtained between the peak area of the drug and the corresponding concentration.

Precision:

Intra day and Inter day precision was determined by repeating assay three times on same day for intra day and on different days for inter day precision

Accuracy:

To check the accuracy of the proposed method, recovery studies were carried out at 80, 100 and 120% of the test concentration as per ICH guidelines. The results of the recovery studies and its statistical validation data indicate high accuracy of the proposed method.

Robustness:

To evaluate the robustness of the developed method, deliberate variations were made in the method parameters such as the flow rate, ratio of mobile phase and the column temperature.

Specificity:

The specificity of the HPLC method was ascertained by analyzing standard drug and sample solutions. The retention time of Aceclofenac and Tizanidine was confirmed by comparing the retention time with that of the standard.

LOD and LOQ:

The LOD and LOQ were separately determined based on the standard deviation of response of thecalibration curve. The residual standard deviation of the regression lines and slope of the calibration curves were used to calculate the LOD and LOQ.

Conclusion

In conclusion, the proposed RP-HPLC method provided a simple, accurate and reproducible method for routine in vitro tests of Aceclofenac and Tizanidine. in dosage forms. Only one method is now availble for the simultaneous estimation of the drugs using spectrophotometry. This is the first report for the simultaneous estimation of Aceclofenac and Tizanidine using HPLC. The short chromatographic time makes this method suitable for the processing of multiple samples in a limited amount of time.

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