Spectrophotometric method development and validation for estimation of Tizanidine and Aceclofenac in Bulk Drug & Tablet formulation

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Abstract: The present study deals with UV spectrophotometric method development & validation for estimation of Tizanidine & Aceclofenac in bulk drug & tablet dosage form by viedort's method & first order UV derivative spectrophotometry. The vierodt's method involves measurement of absorbance at λmax of Tizanidine & Aceclofenac at 318 nm & 274 nm respectively. The linearity of Tizanidine & Aceclofenac was found to be in the range 0f 1-10 μg/ml & 2-20 μg/ml respectively. The % recovery of Tizanidine & Aceclofenac was found out to be 99.72 % & 99.69 %s respectively. First order UV derivative spectrophotometry (D_1 method), the zero crossing method was chosen as Tizanidine could be easily analyzed without any interference from Aceclofenac and vice-versa. Tizanidine was determined by measurement of its D_1 amplitude at the zero crossing point of Aceclofenac at (313 nm), While Aceclofenac was determined by measurement of its D_1 amplitude at zero crossing point of Tizanidine at (250 nm) The proposed method was validated as per ICH guidelines.

Keywords: Tizanidine, Aceclofenac, Vierodt's method, First order derivative method.

Introduction:

Tizanidine HCL [64461-82-1] [TZN] chemically is 5-chloro-N-(2-imidazolin-2-yl)2,1,3-benzothiadiazol-4-yl-amine. Tizanidine is a short acting drug for the management of spasticity. It is an agonist at a 2-adrenergic receptor sites & presumably reduces spasticity by increasing presynaptic inhibition of motor neurons. Aceclofenac [89796-99-6] [ACF] chemically is 2-[2-[2-(2,6-Dichlorophenyl) amino phenyl]cetyl] oxyacetic acid which is used as an effective NSAID having pronounced analgesic, antipyretic, anti-inflammatory property. It is belonging to developed NSAIDS of arylacetic acid type & structurally related to diclofenac. Aceclofenac-tizanidine combination is more effective than aceclofenac alone and had a favourable safety profile in the treatment of acute low back pain and for rheumatic disorders. Aceclofenac is reported for spectrophotometric, RP-HPLC and simultaneous estimation with other combinations[1,3,5,11] Similarly, Tizanidine also reported in combination with other drugs[2,4,6,7,8]. Since no spectrophotometric method is reported for simultaneous estimation of Aceclofenac and Tizanidine in combination using methanol & phosphate buffer. Therefore, the present work, a successful attempt has been made to estimate both these drugs simultaneously by two simple UV spectrophotometric methods (Viedort's method and First order derivative method). The present paper describes a simple, accurate and precise method for simultaneous estimation of Aceclofenac and Tizanidine in combined tablet dosage form. TZN & ACF is official in Indian Pharmacoepoeia 2007 respectively[12].
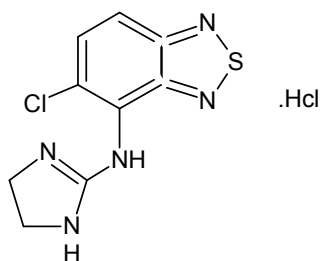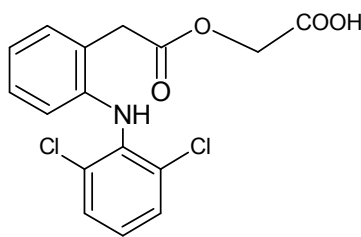
Tizanidine HCL   Aceclofenac

Experimental Method:

Solubility Studies:
Tizanidine is soluble in water but aceclefenac is insoluble in water, hence various solvent systems were screened for simultaneous determination and methanol & phosphate buffer was selected as solvent system.

Instrumentation:
The instrument used was Jasco double beam UV/Vis spectrophotometer model V- 530. Weighing was done on electronic balance (Contech precision balance CB-series).

Materials:
TZN HCL drug sample was kindly supplied by endoc pharma laboratories limited Rajkot (Gujarat, India) and ACF drug sample was supplied by inventia health care (Mumbai, India) and were used without any further purification. AR grade methanol was purchased from merck chemicals, India. Assay was carried out on Acemiz-xl tablet dosage form labeled to contain 2 mg of TZN and 100 mg of ACF.

Preparation of Standard Stock Solutions:
Standard stock solutions of TZN and ACF were prepared separately by dissolving 100 mg of each drug in 10ml of methanol to get standard stock solution of 1000 µg/ml respectively by sonicating for 15 min and 1 ml was pipette out and further volume was made up to 10 ml with phosphate buffer to obtain concentration of 100 µg/ml. Further dilutions were made in phosphate buffer from stock solution to get concentrations of 1-10 µg/ml of TZN & 2-20 µg/ml of ACF. The standard solutions of both TZN & ACF were scanned in the range of 400-200 nm against solvent phosphate buffer and spectra was recorded. λmax of TZN & ACF was found at 318 nm & 274 nm respectively.

Procedure:
From the stock solution of 100 µg/ml working standard solutions of drug were prepared by appropriate dilutions were prepared in phosphate buffer, were scanned in entire UV range to determine λmax. TZN has λmax of 318 nm while ACF has λmax of 274 nm respectively. Standard stock solution were prepared having concentration of 1-10 µg/ml of TZN & 2-20 µg/ml of ACF. The absorbance of these standard stock solution were measured at 318 nm & 274 nm and calibration curve were plotted.

The standard solutions were prepared individually with methanol & further dilutions were prepared in phosphate buffer for spectrophotometric measurements. The zero order spectra were recorded over 200-400 nm for TZN and ACF. As shown (Fig.1), the zero order spectra of pure drugs were found to be non overlapping, making simultaneous determination difficult. In contrast, the first derivative spectra of TZN and ACF showed zero crossing points (Fig.2). The shape of the first derivative spectra is adequate for determining TZN in the presence of ACF and vice versa. TZN was determined by measurement of its D1 amplitude at the zero crossing point of ACF at (313nm), While ACF was determined by measurement of its D1 at zero crossing point of TZN at (274 nm).
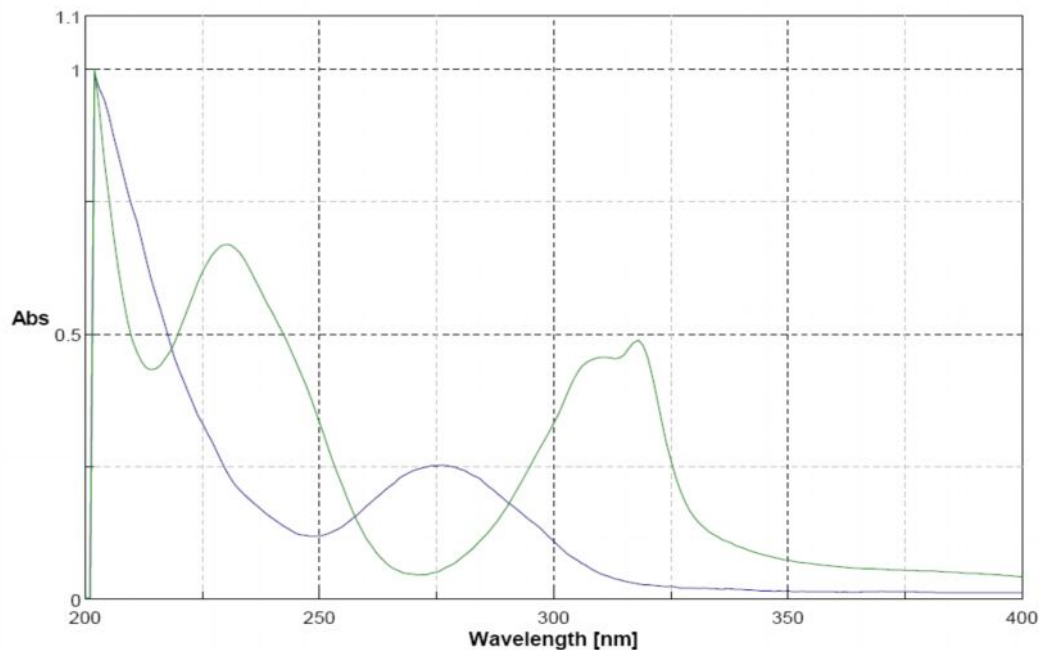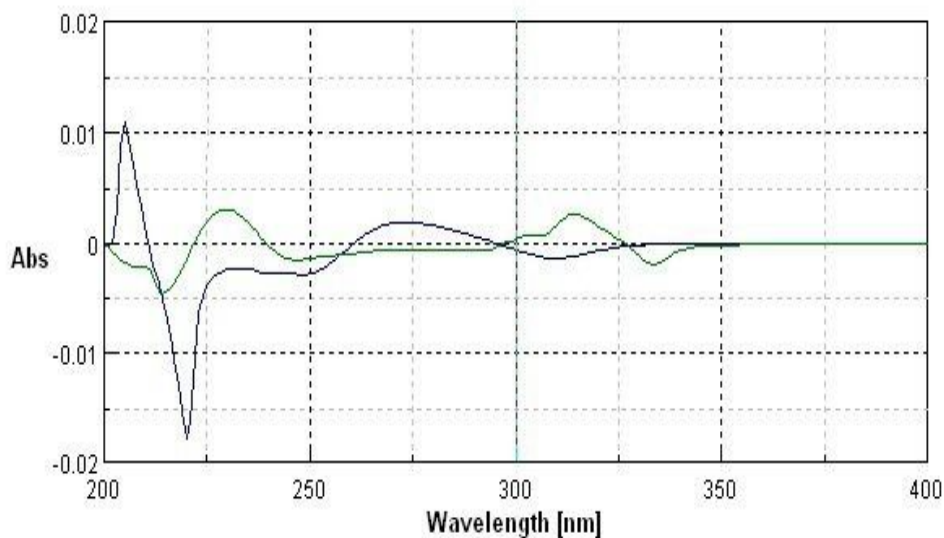
Fig. 1 Overlain Zero order spectra of TZN and ACF

Fig. 2:- Overlain first derivative spectra of TZN & ACF.

Procedure for the Analysis of Tablet Formulation:

Ten tablets containing label claim of 2 mg of TZN and 100 mg of ACF were weighed and finely powdered. Weight of the powder equivalent to 0.0225 mg tablet was accurately weighed, transferred into a 100 ml flask, dissolved in methanol and this solution was sonicated for about 20 minutes filtered to separate any insoluble matter and volume was made up to 100 ml with phosphate buffer. The clear solution obtained was diluted to get appropriate concentration in linearity ranges and absorbances were measured.

Recovery Studies:

To study the accuracy of the proposed method, recovery studies were carried out at three different levels 80%, 100% and 120% by addition of known amount of TZN and ACF to a known concentration of the commercial tablet.

TABLE 1: VALIDATION AS PER ICH GUIDELINES

Parameters	Method I		Method II	
	TZN (318nm)	ACF (274nm)	TZN (318nm)	ACF (274nm)
Linearity range(µg/ml)	1-10 µg/ml	2-20 µg/ml	1-10 µg/ml	2-20 µg/ml
Correlation coefficient (r^2)	0.999	0.998	0.996	0.997
Interday	0.30	0.07	0.43	0.09
Intraday	0.61	0.05	0.74	0.10
Slope	0.0344	0.1052	0.0317	0.3563
Intercept	0.0017	0.0003	0.0016	0.0004

TABLE 2 : RESULT OF RECOVERY STUDIES

Level of % recovery	% mean recovery [*]		Standard deviation		% RSD	
	TZN	ACF	TZN	ACF	TZN	ACF
80	98.66	98.83	1.01	0.9	0.216	0.227
100	99.72	99.60	1.02	1.10	0.255	0.286
120	100.72	101.28	1.12	1.5	0.320	0.406

*average of six determinations

Results and Discussion:

The present work provides an accurate rapid, sensitive method for the simultaneous analysis of TZN & ACF in bulk and tablet formulation. Linear relationships between D_1 amplitude and drug concentration were obtained over the range of at 1-10 & 2-20 µg/ml for TZN and ACF respectively and also by simultaneous estimation respectively. The correlation coefficient, slope and intercept obtained for each drug is as shown in Table1. The proposed method was also successfully applied to a pharmaceutical formulation. The % assay was found to be 99.63% for TZN and 99.07% for ACF. No interference was observed from the pharmaceutical adjuvants. Recovery studies results are tabulated in Table 2. For TZN percent recovery ranged from 98.66 % to 100.72 %, with % RSD ranging from 0.216 % to 0.320 %. For ACF, percent recovery ranged from 98.83 % to 101.28 %, with % RSD ranging from 0.227 % to 0.406 %. Hence, the proposed method was evaluated statistically and was validated in terms of precision, linearity and accuracy.

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