

SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF ACECLOFENAC AND TIZANIDINE

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ABSTRACT: Two simple spectrophotometric methods have been developed for simultaneous estimation of Aceclofenac and Tizanidine from tablet dosage form. Method I simultaneous equation method involves the measurement of absorbances at two wavelengths 274 nm (λ_{max} of Aceclofenac) and 319 nm (λ_{max} of Tizanidine). Method II involves Absorbance correction method in which absorbance is measured at two wavelengths 274 nm at which Tizanidine has no absorbance and 319 nm at which both the drugs have considerable absorbance. The method was found linear between the range of 5- 25 $\mu\text{g/ml}$ for Aceclofenac and 2-10 $\mu\text{g/ml}$ for Tizanidine for both the methods. The accuracy and precision were determined and validated statistically. Both the methods showed good reproducibility and recovery with % RSD less than 1. The methods were found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of Aceclofenac and Tizanidine in bulk and combined dosage form.

KEY WORDS: Aceclofenac, Tizanidine, Absorbance Correction method, Simultaneous Equation method.

INTRODUCTION

Aceclofenac, {2, (2,6dichlorophenyl) amino phenyl acetic acid.}, is a phenyl acetic acid derivative with the improved gastric tolerance and is used for relief pain and inflammation in rheumatoid arthritis¹. Tizanidine is chemically {5, chloro N- (2 imidazolyl) 2, 1, 3, benzothiadiazol 4- yl amine hydrochloride}, is centrally acting skeletal muscle relaxant with central analgesic effect and gastric tolerance effect with combination on NSAIDs². Aceclofenac-tizanidine combination was 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 spectrophotometric³, RP-HPLC⁴ and simultaneous estimation with other combinations^{5,6}. Similarly, Tizanidine also reported in combination with other drugs⁷⁻¹⁶. Since no spectrophotometric method is reported for simultaneous estimation of Aceclofenac and Tizanidine in combination therefore, in the present work, a successful attempt has been made to estimate both these drugs simultaneously by two simple UV

spectrophotometric methods (simultaneous equation method and absorbance correction method).

The present paper describes a simple, accurate and precise method for simultaneous estimation of Aceclofenac and Tizanidine in combined tablet dosage form.

MATERIALS AND METHODS

Instrument used is an UV-Visible double beam spectrophotometer, Make: Elico (model SL 164) with 1cm matched quartz cells. Working standards of Aceclofenac and Tizanidine were obtained as gift sample from Healthcare Pharmaceuticals, Pondicherry, India. Ethanol (95%) was used as solvent. Combined dose tablet formulation (ASMR) was procured from local market.

PREPARATION OF STOCK SOLUTION:

Accurately weighed quantity of Aceclofenac (100 mg) and Tizanidine (100 mg) was transferred to two separate 100 ml volumetric flasks, dissolved in 1mL

methanol and diluted to the mark with the methanol (stock solution:1000 µg/ml).

SIMULTANEOUS EQUATION METHOD (OR) VIERODT'S METHOD (METHOD -I):

From the stock solution of 1000 µg/ml, working standard solutions of drugs were prepared by appropriate dilution and were scanned in entire UV range to determine λ_{\max} . Aceclofenac has λ_{\max} of 274 nm while Tizanidine has λ_{\max} at 319 nm respectively (Fig.1). Standard solutions were prepared having concentration of 5-25 µg/ml for Aceclofenac and 2-10 µg/ml for Tizanidine. The absorbances of these standard solutions were measured at 274 and 319 nm and calibration curves were plotted. Two simultaneous equations were formed using these Absorptivity coefficient values.

$$A_1 = (0.03956) C_1 + (0.0044) C_2 \text{ ----- 1}$$

$$A_2 = (0.00036) C_1 + (0.0572) C_2 \text{ ----- 2}$$

Where, C_1 and C_2 are the concentrations of Aceclofenac and Tizanidine measured in µg/ml, in sample solutions. A_1 and A_2 are the absorbances of mixture at selected wavelengths 274 nm and 319 nm respectively.

By applying the Cramer's rule, the concentration C_{ACN} and C_{TZN} , can be calculated as follows,

$$C_{ACN} = \frac{A_2 (0.0044) - A_1 (0.0572)}{0.02261248} \text{ ----- 3}$$

$$C_{TZN} = \frac{A_2 (0.00036) - A_1 (0.03956)}{0.02261248} \text{ ----- 4}$$

ABSORBANCE CORRECTION METHOD (METHOD II):

Absorbance correction method uses the absorbances at two selected wavelengths, one at λ_{\max} of one drug where other drug also shows considerable absorbance and other being the wavelength at which the first drug has practically nil absorbance. From the stock solutions, working standard solutions of Tizanidine (10µg/ml) and Aceclofenac (10µg/ml) were prepared by appropriate dilution and were scanned in the entire UV range to determine the wavelengths. Aceclofenac and Tizanidine have λ_{\max} at 274 nm and at 319 nm respectively and absorbance is measured at two wavelengths i.e, 274 nm at which Tizanidine has no absorbance and 319 nm at which both the drugs have considerable absorbance. A series of standard solutions ranging from 5-25 µg/ml for Aceclofenac and 2-10 µg/ml for Tizanidine both were prepared and the absorbance of solutions was recorded at 274 nm and 319 nm to plot a calibration curve of absorbance versus concentration. The calibration curves were found to be linear in the concentration range under study and are presented in Table-1.

The concentration of two drugs in mixture was calculated by using following equations:

$$C_{ACN} = \frac{A_1}{ax1} \text{ ----- (5)}$$

The concentration of the other drug is calculated by using the formula obtained by rearranging the equation 4 i.e.

$$C_{TZN} = \frac{A_2 - ax2Cx}{ay2} \text{ ----- (6)}$$

Where A_1 and A_2 are the absorbances of mixture at 274 nm and 319 nm and $ax1$, $ax2$ and, $ay2$ are absorptivities of two drugs at 274 nm and 319 nm.

Table 1: Validation Parameter

Parameters	Method-1		Method-2	
	ACN 279nm	TZN 319nm	ACN 279nm	TZN 319nm
Linearity Range(µg/ml)	5-25	2-10	5-25	2-10
Coefficient of Correlation	0.9999	0.9998	0.9999	0.9999
Slope	0.0327	0.0419	0.0419	0.0513
-Intercept	0.0015	0.0002	0.0016	0.0005
Repeatability (% RSD)				
Intraday precision	0.71-0.88	0.57-0.93	0.38-0.55	0.62-1.27
Interday precision	0.53-1.12	0.43-1.32	0.81-1.32	0.55-0.94

ACN = Aceclofenac, TZN= Tizanidine

ANALYSIS OF TABLET FORMULATION SIMULTANEOUS EQUATION METHOD

(METHOD –I):

Twenty tablets are weighed and powdered. A quantity equivalent to 50 mg of Aceclofenac and 1 mg of Tizanidine was taken in 50 ml volumetric flask. The Tizanidine present in this tablet powder was 1 mg, which could not be found out accurately due to low absorbance hence to increase the accuracy, accurately weighed 9 mg of pure drug of Tizanidine, was added to the crushed tablet, which increases the amount of Tizanidine to 10 mg. It was then extracted with 10 ml of ethanol 3 times. The extract was filtered through Whatman filter paper, and residue washed with ethanol. The filtered extract and washing were transferred into 50 ml volumetric flask and made up to 50 ml. The filtrate was further diluted to get final concentration of 25 µg/ml of Aceclofenac and 5 µg/ml of Tizanidine. Absorbances of sample solutions were recorded at 274 (λ_{max} of Aceclofenac) and 319 nm (λ_{max} of Tizanidine) and concentration of two drugs in the sample were determined by using equations 3 and 4.

ABSORPTION CORRECTION METHOD

(METHOD II):

Twenty tablets were weighed and powdered .A quantity equivalent to 50 mg of Aceclofenac was taken and dissolved in 50ml ethanol. From the above dilution 12.5 ml was diluted to 50 ml to get 250 µg/ml and 5 µg/ml of Aceclofenac and Tizanidine and absorbance of this mixture was measured at 319 nm (Dilution I). From above dilution I Aceclofenac was further diluted to get 10 µg/ml and absorbance was measured at 274 nm. The concentrations of two drugs in sample were determined by using equation 5 and 6. The analysis procedure was repeated for 6 times with tablet formulations. The result of analysis of tablet formulation is reported in Table 2.

PRECISION:

Precision was checked out by performing interday variation and intraday variation studies. In interday variation the absorbance for standard solution was measured on three consecutive days. In intraday variation the absorbances were measured three times in a day. The results were satisfactory and presented in Table 1.

RECOVERY STUDIES:

To study the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels. A known amount of drug was added to preanalyzed tablet powder and percentage recoveries were calculated. The results of recovery studies were satisfactory and are presented in Table-3.

RESULTS AND DISCUSSION

For both the two methods linearity was observed in the concentration range of 5-25 µg/ml for Aceclofenac and 2-10 µg/ml for Tizanidine. Marketed brand of tablet was analyzed and amount of drug determined by proposed methods ranges from 99.26 to 101.03% as shown in Table 2. The proposed methods were validated as per ICH guideline. The accuracy of method was determined at 80, 100 and 120 % level. The % recovery ranges from 99.14 to 101.43 for both the two methods. Precision was calculated as interlay and intraday variations (%RSD is less than 1.5) for both drugs. The proposed methods were found to be simple, accurate and rapid for the routine determination of Aceclofenac and Tizanidine in tablet formulation. The two methods can be successfully used for simultaneous estimation of Aceclofenac and Tizanidine in combined dosage form.

Table 2 : Results of Analysis of Marketed Formulation for Method I & II

Method	Label claim		% of label claim estimated± RSD*	
	ACN (mg)	TZN (mg)	ACN	TZN
Method-I	100	2mg	99.98±0.987	101.03±1.534
Method-II	100	2 mg	99.60±1.125	99.26±0.258

*RSD of Six Determinations ACN = Aceclofenac, TZN= Tizanidine

Table 3: Results for Recovery Studies

Recovery level	Method I				Method II			
	Amt. of drug added (mg)		% RSD Recovery *		Amt. of drug added (mg)		% RSD Recovery *	
	ACN	TZN	ACN	TZN	ACN	TZN	ACN	TZN
80%	20	4	100.18 \pm 0.147	100.73 \pm 0.997	8	4	99.92 \pm 0.095 2	101.22 \pm 0.452
100%	25	5	98.26 \pm 0.134	100.83 \pm 0.123	10	5	99.82 \pm 0.521	101.43 \pm 0.869
120%	30	6	100.33 \pm 0.547	100.18 \pm 1.203	12	6	100.56 \pm 0.65 8	99.14 \pm 0.125

*RSD for Six Determinations

ACN = Aceclofenac, TZN= Tizanidine

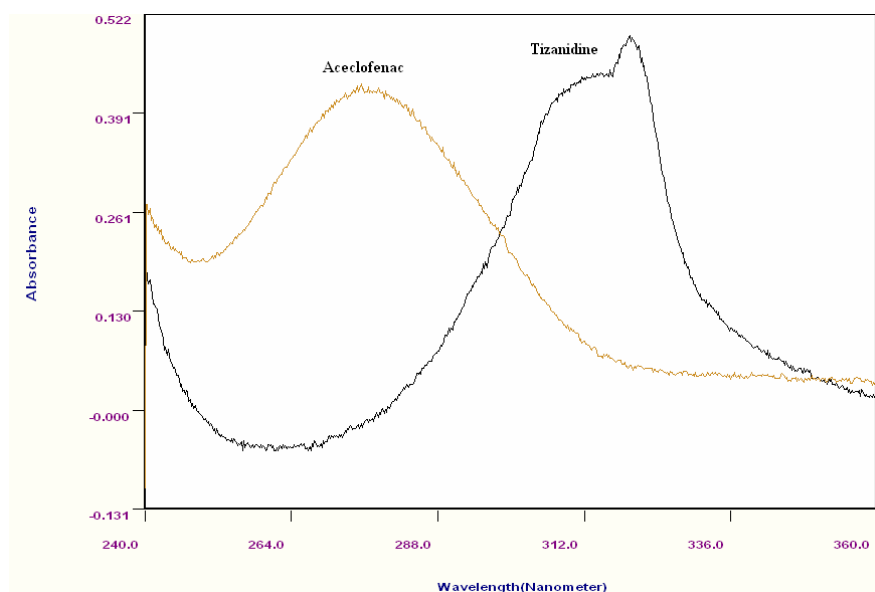


Figure -1 Overlain Spectra Of Tizanidine And Aceclofenac

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