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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DICLOFENAC AND TIZANIDINE IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

An isocratic, reversed phase-liquid-chromatographic method was developed for the quantitative determination of Tizanidine and Diclofenac sodium combined-tablet dosage form. A Inertsil ODS column (250*4.6mm, 5µm) with mobile phase containing Potassium dihydrogen phosphate buffer[pH 6.9]:ACN(55:45) v/v) was used. The flow rate was 1.0mL/min, column temperature was 30°C and effluents were monitored at 228nm.The retention times of Tizanidine and Diclofenac sodium were 2.517 min and 4.223 min, respectively. The correlation co-efficient for Tizanidine and Diclofenac sodium was found to be 0.99 and 0.99, respectively .The proposed method was validated with respect to linearity, accuracy, precision, specificity and robustness. Recovery of Tizanidine and Diclofenac sodium formulations was found to be in the range of 97-103% and 97-103% respectively confirms the non-interferences of the excipients in the formulation. Due to its simplicity, rapidness and high precision. The method was successfully applied to the estimation of Tizanidine and Diclofenac sodium in combined dosageform.

KEYWORDS: RP-HPLC, Tizanidine and Diclofenac sodium.

INTRODUCTION

TIZANIDINE is a widely used Analgesic anti biotic and belongs to α-adrenergic agonist. Its chemical formula is 5-chloro-N-(2-imidazolin-2yl)-2,1,3-benzothiadiazol-4-yl amine.

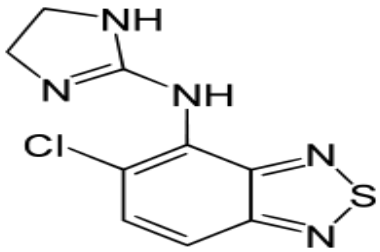

Fig no:1 Tizanidine

Tizanidine reduces spasticity by increasing presynaptic inhibition of motor neurons through agonist action at α-2 adrenergic receptor sites.

Diclofenac Sodium is widely used analgesic and belongs to Non steroidal Anti-inflammatory drug (NSAID).Its chemical formula is 2-[(2,6-Dichlorophenyl)-amino] phenyl acetate.

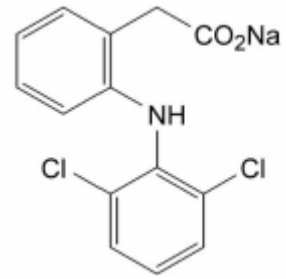

Fig no:2 DICLOFENAC SODIUM

The anti inflammatory effect of diclofenac are believed to be due to inhibition of both leucocyte migration and the enzyme cyclooxyginase leading to the peripheral inhibiition of prostaglandil synthesis. As prostaglandin sensitize pain receptors, inhibition of their synthesis is responsible for the analgesic effect of diclofenac. Antipyrutic effects may be due to action of the hypothalamus, resulting in peripheral dilation, increased coetaneous blood flow and subsequent heat dissipation.

MATERIAL AND METHODS

Instrumentation

SHIMADZU HPLC instrument with Inertsil ODS column(250×4.6mm, 5µm) and Spinchrom software were used to develop the method for the quantitative estimation of Diclofenac and Tizanidine. A 1µL auto sampler injector unit, sonicator, GLOBAL Digital pH meter(DPH-500), Electronic analytical balance(SHIMADZU AY220 Max d=0.1mg), UV-VIS Spectrophotometer (ELECTRON CORPORATION, NICOLET evolution 100) were also used.

Chemicals and Reagents

HPLC grade analytical Acetonitrile, HPLC grage Analytical Methanol, Potassium Dihydrogen Phosphate, Phosphoric acid, TriEthylamine, HPLC grade analytical water were used.

HPLC Conditions

Acetonitrile (HPLC grade) and mobile phase consisting of Potassium dihydrogen phosphate buffer[pH 6.9]and adjust the pH 3.5 with OPA were filtered through 0.45µ membrane filter prior to use. before pumping from the solvent reservoir they were degassed. in the ratio of 30:70v/v were pumped into the column at a flow rate of 1.0ml/min and ambient temperature. The detection was monitored at 228nm and the runtime was 6min. volume of injection loop was 10µl .prior to injection of the drug solution, the column was equilibrated for about 15min.with the mobile phase flowing through the system.

Preparation of standard solution of the drug

Diclofenac(10 mg) and Tizanidine(10mg) working standards were accurately weighed and transfered into separate, thoroughly cleaned and dried volumetric flasks, diluted with methanol sonicated for 10minutes and then finally made up to the final volume with diluent to obtain 1000ppm per mL concentrated solution.

Specificity

It is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. In general, these might include impurities, matrix, degradants etc.

Accuracy and Precision

The methods accuracy and precision was determined by recovery experiments the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained. The recovery studies were conducted six times for precision.

Preparation of sample solution

The analysis of drugs, 20 tablets were weighed and triturated in glass mortar and quantity of powder equivalent to 10mg of Tizanidine was transferred to 10ml volumetric flask and dissolved in sufficient quantity of methanol. It was sonicated for 10min and volume was made up to 10ml to obtain a stock solution of 1000µg/ml of tizanidine and 2500µg/ml. This solution was then filtered through nylon 0.45mm membrane filter. It was further diluted with

mobile phase to get the required test concentrations of 4 µg/ml of TIZ and 120µg/ml of Diclofenac. This solution was injected 6 times in to the column and chromatograms were recorded and respective peak areas were measured. The contents of TIZ and Diclofenac were calculated by using the regression equation.

Method Validation

System Suitability Studies: The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table1).the suitability of the system for the analysis of this drug combinations was demonstrated by the values obtained .the system suitability parameters may fall within ±3% standard deviation range during routine performance of the method. Standard drugs added recoveries were found to be accurate (Table-3). inter-day and intra-day variation method was used to demonstrate precision of the studies done .six repeated injections in intraday studies of standard and sample solutions revealed the response factor of drug peaks and percentage RSD .In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated .chromatograms of three different levels were shown in Fig3, the data obtained proved the developed Rp-hplc method to be precise (table -2).

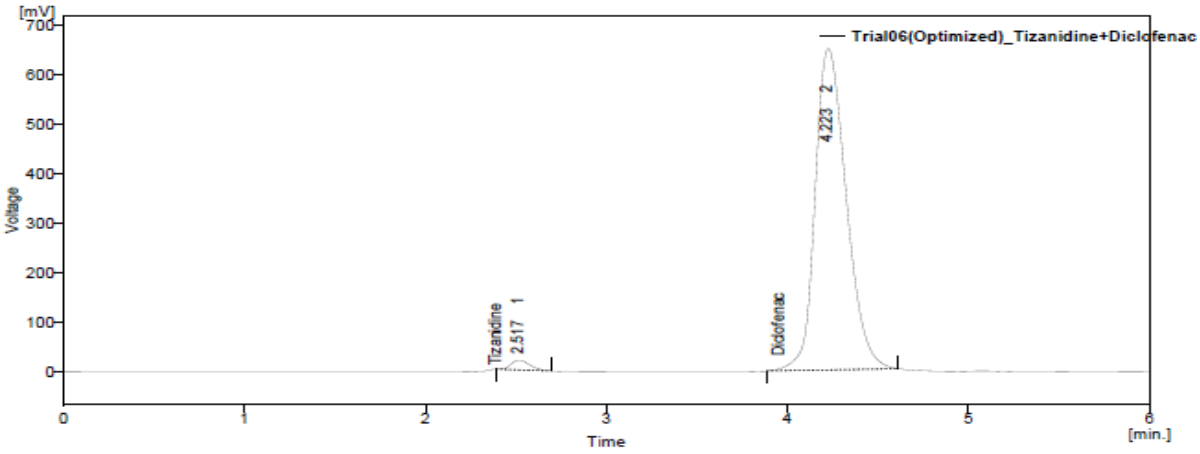

Fig.3 Standard Chromatogram For Diclofenac And Tizanidine

Table1: System Suitability Parameters

PARAMETERS	DICLOFENAC	TIZANIDINE
Correlation Coefficient	0.9965	0.9993
LOD	6.27	0.004
LOQ	18.99	0.012
Theoretical plates	6590	3473

Table 2: Precision studies of Diclofenac and Tizanidine

Amount of std taken (µg/ml)	Intra-day precision (n=3)		Inter-day precision (n=3)	
	Mean±SD	%RSD	Mean±SD	%RSD
Diclofenac	7809.627±61.288	0.75	7810.529±61.503	0.76
Tizanidine	146.140±2.112	1.45	150.021±2.122	1.45

Accuracy

The accuracy of an analytical method is the closeness of that results obtained by that method to the true value. Accuracy may often be expressed as percent recovery by the assay of known added amount of analyte. The accuracy of the method was determined by the standard addition method. A known amount of the standard drug was added to the fixed amount of pre-analyzed tablet solution. The standard addition method was performed at 100%, 120% and 140% levels. The solutions were analysed in triplicate at each level as per the proposed method. The %RSD and recoveries were obtained within the range, results were shown in table 3.

Table-3: Recovery Report of Diclofenac and Tizanidine

Drug	Pre analysed Conc. Taken (µg/ml)	Recovery Level	Amount of Drug Added (µg)	Amountof Drug Found (µg/ml) Mean±S.D(n=6)	% Recovery	Acceptance Criteria
DICLO	20	100%	80	99.98	99.98	98-102%
		120%	100	118.49	98.74	
		140%	120	142.70	101.93	
TIZ	0.8	100%	3.2	4.01	100.36	98-102%
		120%	4.0	4.81	100.25	
		140%	4.8	5.59	99.81	

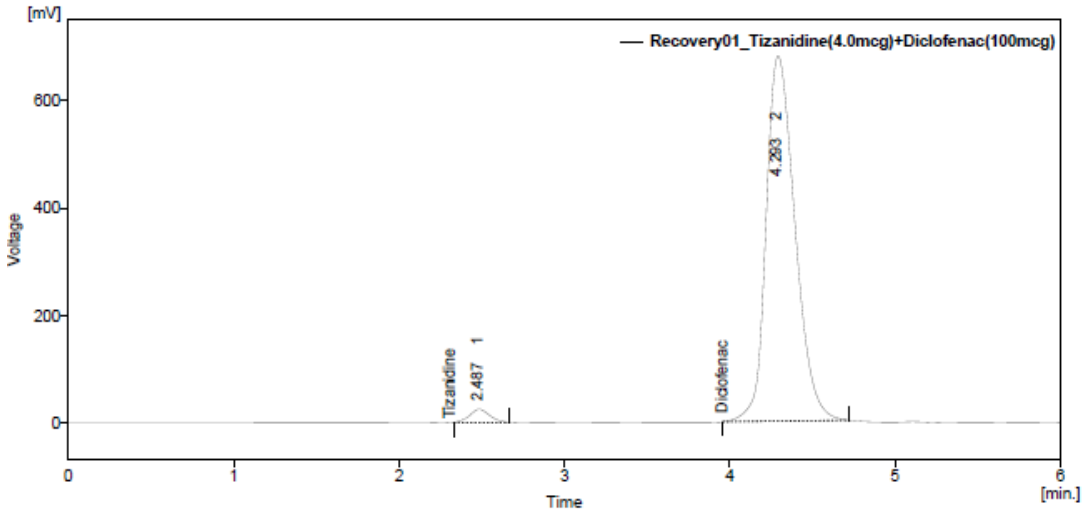

Fig.4: 100% Recovery level of TIZ and Diclofenac

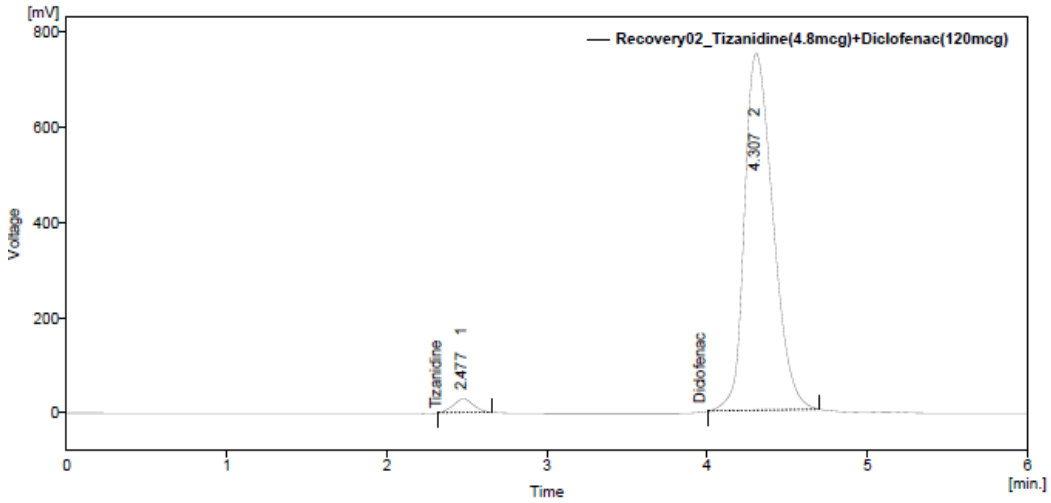

Fig.5:120% Recovery level of TIZ and Diclofenac

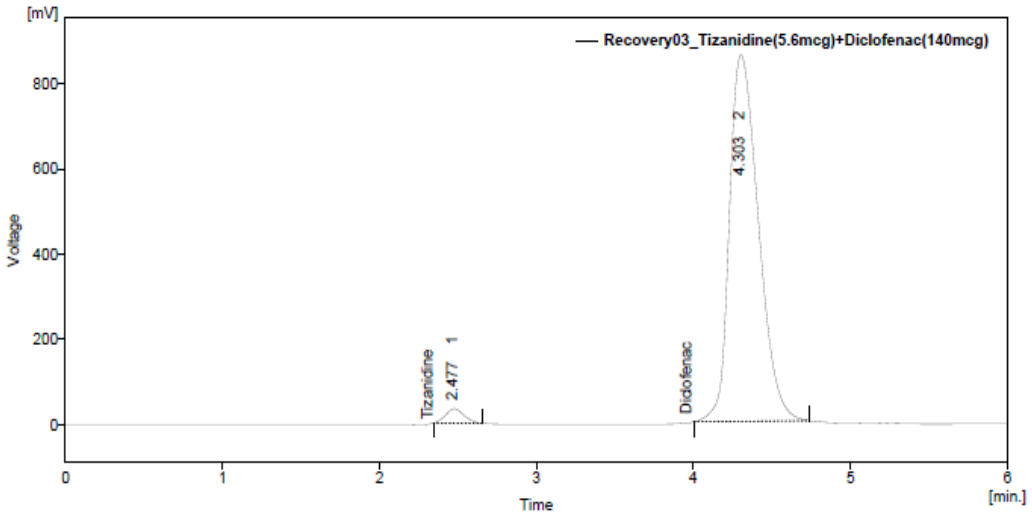

Fig.6: 140% Recovery level of Tizanidine and Diclofenac

Linearity and Range

Linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 66.632x + 1188.5 (R^2=0.99) for Diclofenac and y = 65.45x - 70.876 for Tizanidine The results show an excellent correlation existed between areas and concentration of drugs within the concentration range as indicated above .The overlay chromatograms of Linearity for Diclofenac and Tizanidine were shown inFig6 and results for calibration curves are given in fig 7&8.

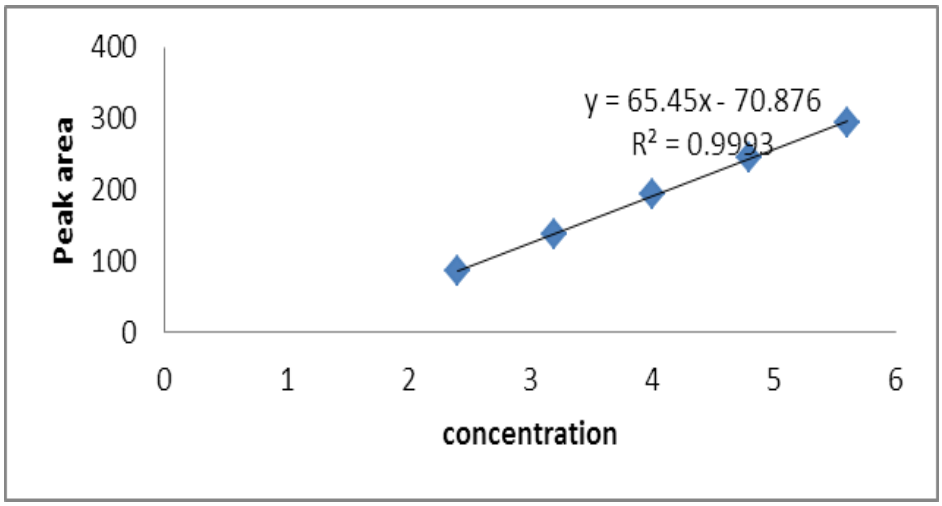

Fig-7: Linearity graph of Tizanidine Hydrochloride.

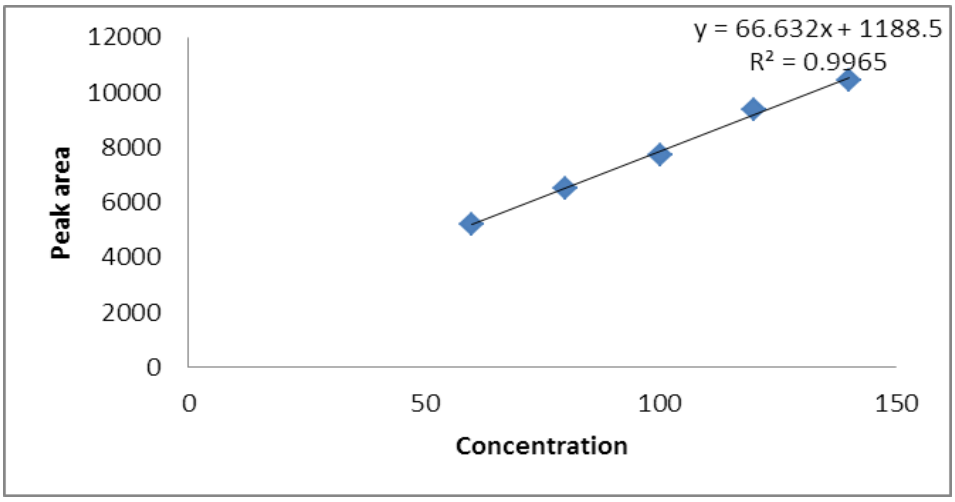

Fig-8: Linearity graph of Diclofenac sodium.

Robustness

Robustness of the current method was investigated by analyzing the standard solution and established system suitability with the deliberate variation of mobile phase organic variation, flow rate and column temperature. (Table-4).

Table-4: Robustness study results of Diclofenac and Tizanidine by RP-HPLC.

Parameter	Condition	R_t (in min)		Remark
		Diclo	TIZ	
Optimised	1ml/min, 55:45, P_H 3.5	4.223 min	2.517 min	**Robust**
Flow rate	0.8ml/min	6.293	4.110	Robust
	1.2ml/min	3.203	2.087	Robust
Wavelength	278 nm	4.257	2.803	Robust
	282 nm	4.253	2.793	Robust

CONCLUSION

As the solvent is economical and the method were simple, accurate and precise, these methods can be for routine analysis of Tizanidine hydrochloride and Diclofenac sodium in bulk and formulation. In this proposed HPLC methods the selected drugs showed good linearity, recovery studies are within the limits(98-102%), sample recoveries were in good agreement with their true values and the suggested non-interference of excipients in the estimation, mobile phase and solvents are simple to prepare and economical , reliable and less time consuming. So it can be decided that the simple, precise, accurate, specific, economical and short proposed methods were found to be most useful for analysis purpose.

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