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Simultaneous Estimation of Tizanidine and Valdecoxib in Combined Dosage Forms by RP-HPLC Method

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A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed for the simultaneous estimation of tizanidine and valdecoxib from tablets. The process was carried out on a Phenomenex LUNA C_{18} (25 cm × 4.6 mm i.d. 5 μ) column with a mobile phase consisting of acetonitrile : 0.5% triethylamine (adjusted to pH 3.0 using orthophosphoric acid) (50 : 50 v/v) at a flow rate of 1.0 mL/min. Detection was carried out at 240 nm. nimesulide was used as internal standard. The retention time of nimesulide, valdecoxib and tizanidine was 6.04, 8.95 and 10.34 min respectively. The validation of the proposed method was also carried out. The proposed method can be used for the estimation of these drugs in combined dosage forms.

Key Words: Tizanidine, Valdecoxib, HP-HPLC Estimation.

INTRODUCTION

Valdecoxib is chemically designated as 4-(5-methyl-3-phenyl-4-isoxazolyl) benzene sulfonamide and is a diaryl substituted isoxazole. Valdecoxib is a nonsteroidal anti-inflammatory drug (NSAID) that exhibits antiinflammatory, analgesic and antipyretic properties. Tizanidine is chemically 5-chloro-4-(2-imidazolin-2-ylamino)-2,1,3-benzothiodiazole[1]. It is a muscle relaxant (skeletal). Many methods have been described in literature for the determination of valdecoxib and tizanidine individually and in combination with other drugs[2–12]. However there is no HPLC method reported for the simultaneous estimation of these drugs in combined dosage forms. A fixed dose combination containing tizanidine 2 mg and valdecoxib 20 mg is available in tablet form in the market. The present work describes a simple, precise and accurate reversed phase HPLC method for the simultaneous estimation of tizanidine and valdecoxib in combined dosage forms.

EXPERIMENTAL

Acetonitrile HPLC grade was procured from E. Merck (India) Ltd., Mumbai. Triethylamine and orthophosphoric acid AR grade were procured from Qualigens

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Fine Chemicals, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system. Reference standards of valdecoxib and tizanidine were procured from Unichem Pharmaceuticals, Mumbai and nimesulide was procured from Cadilla Pharmaceuticals Ltd., Ahmedabad.

Chromatographic conditions

A Shimadzu® HPLC (LC-10AT *VP*) system was used for the analysis. The process was carried out on Phenomenex LUNA C_{18} (25 cm × 4.6 mm i.d., 5 μ) column as a stationary phase and acetonitrile : 0.5% triethylamine (adjusted to pH 3.0 using orthophosphoric acid) (50 : 50 v/v) as the mobile phase at a flow rate of 1.0 mL/min. Rheodyne 7725*i* injector with 20 μL loop was used for the injection of samples. Detection was done at 240 nm. The mobile phase was filtered through 0.2 μ membrane filter and degassed.

Preparation of solutions

Standard stock solutions of 1 mg/mL of tizanidine and valdecoxib were prepared separately using a mixture of water and acetonitrile (1 : 1 v/v); from the standard stock solution, mixed standard solution was prepared to contain 0.2 μg/mL of tizanidine, 2 μg/mL of valdecoxib and 5 μg/mL of nimesulide as internal standard.

Twenty tablets, each containing 2 mg of tizanidine and 20 mg of valdecoxib were weighed and finely powdered. A quantity of powder equivalent to 0.2 mg of tizanidine and 2 mg of valdecoxib was weighed and transferred to a sintered glass crucible. To this 5 mL of 1 mg/mL of nimesulide was added and the drugs were extracted with three quantities, each 20 mL, of mixture of acetonitrile and water (1 : 1 v/v). The combined extracts were made up to 100 mL with mobile phase and further dilutions were made to get a concentration of 0.2 μg/mL of tizanidine, 2 μg/mL of valdecoxib (theoretical value) and 5 μg/mL of nimesulide as internal standard and this solution was used for the estimation.

Assay method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of nimesulide, valdecoxib and tizanidine was found to be 6.04, 8.95 and 10.34 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factors (peak area ratio of standard peak area and internal standard peak area) of the standard solution and sample solution were calculated (Table-2). The concentrations of the drugs were calculated (Table-1) using the following formula:

$$\text{Concentration of drugs} = \frac{\text{Response factor of the sample}}{\text{Response factor of the standard}} \times \text{Concentration of standard}$$

TABLE-1
RESULTS OF ANALYSIS OF FORMULATION AND RECOVERY STUDIES

Drug	Amount (mg/tab)		% Label claim*	% Recovery*
	Label claim	Found ± SD*		
Tizanidine	2	1.9675 (±0.0156)	98,38 (±0.7801)	99.46 (±0.8355)
Valdecoxib	20	19.7869 (±0.2158)	98.94 (±1.0788)	100.12 (±0.4121)

*Average of 6 determinations.
Formulation I, ZULU-V (Unichem Pharmaceuticals), each tablet containing 2 mg of tizanidine and 20 mg of valdecoxib.

TABLE-2
LINEARITY AND RANGE

Internal standard peak area (5 µg/mL nimesulide)	Tizanidine			Valdecoxib		
	Concentration (µg/mL)	Peak area	Response factor	Concentration (µg/mL)	Peak area	Response factor
532673	0.1	12351	0.023	1.0	104378	0.196
	0.2	24703	0.046	1.5	156487	0.294
	0.3	37043	0.070	2.0	208616	0.392
	0.4	49394	0.093	2.5	261295	0.491
	0.5	61755	0.116	3.0	312835	0.587

Method validation

Accuracy of the method was studied by recovery experiments. To the powdered tablet formulation (0.2 mg of tizanidine and 2 mg of valdecoxib), 5 mL of 1 mg/mL of nimesulide solution and reference standard drugs were added at the level of 25, 50 and 100% of the label claim. The extraction of drugs was followed using sample preparation procedure and these were analyzed. The percentage recovery was calculated and presented in Table-1. Precision of the method was demonstrated by repeatability studies. This was done by injecting consecutively the standard solution 10 times and passing them through the assay procedure.

Linearity and range of the method was determined by analyzing mixed standard containing 0.1–0.5 µg/mL of tizanidine and 1.0–3.0 µg/mL of valdecoxib (50 to 150% of targeted level of the assay concentration) containing 5 µg/mL of nimesulide as internal standard respectively. The calibration curve was plotted using response factor *vs.* concentration of standard solution; the values are presented in Table-2. The limit of detection (LOD) and limit of quantification (LOQ) of the method were determined by injecting progressively low concentrations of the standard solutions with the optimized chromatographic conditions.

RESULTS AND DISCUSSION

The chromatograms of sample solutions are presented in Fig. 1. The accuracy of the method was determined by recovery studies were carried out and the percentage recovery was calculated. From the data obtained, recoveries for the standard drugs were considered accurate. The precision procedure was satisfactory. The concentration ranges from 0.1–0.5 μg/mL for tizanidine and 1.0–3.0 μg/mL for valdecoxib were examined by the assay procedure and the calibration curves were plotted. The calibration curve shows linear response over the range of concentration used in the assay procedure. The calibration curve passes through the origin, which justifies the use of single point calibration and the proximity of all points to the calibration line demonstrates that the method has adequate linearity to the concentration of the analyte. Calibration curves are shown in Fig. 3.

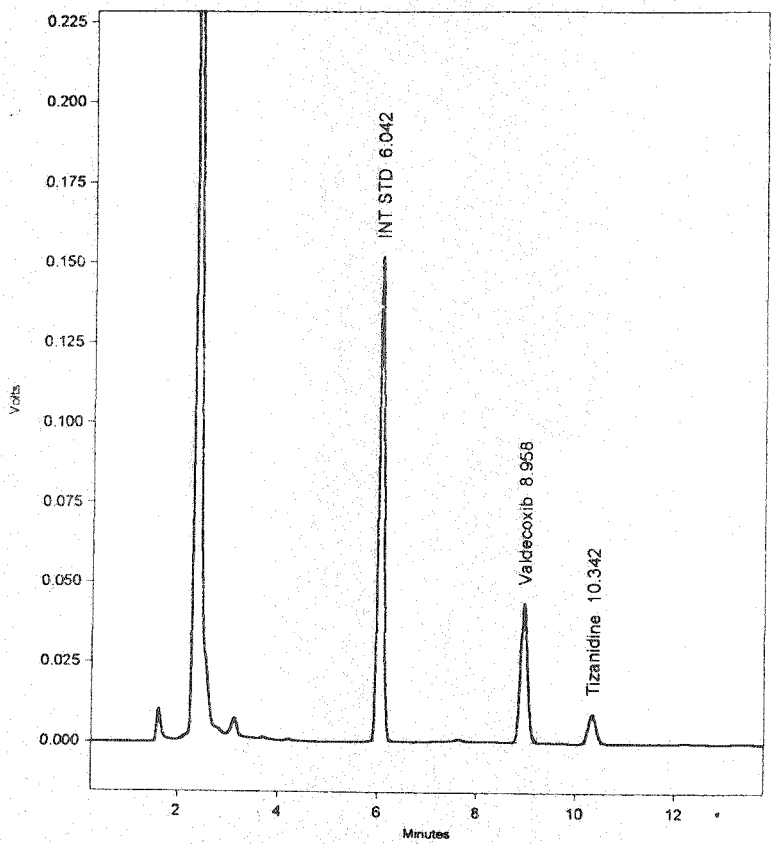

Fig. 1. Typical chromatogram of sample solution

The limits of detection (LOD) for tizanidine and valdecoxib were found to be 10 ng/mL and 5 ng/mL respectively and the limits of quantification (LOQ) were 80 ng/mL and 15 ng/mL for tizanidine and valdecoxib (Table-3). The ruggedness of the method was determined by carrying out the experiment of different instruments like Shimadzu HPLC (LC-10AT), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil, Phenomenex LUNA, and Hichrom. Robustness of the method was determined by

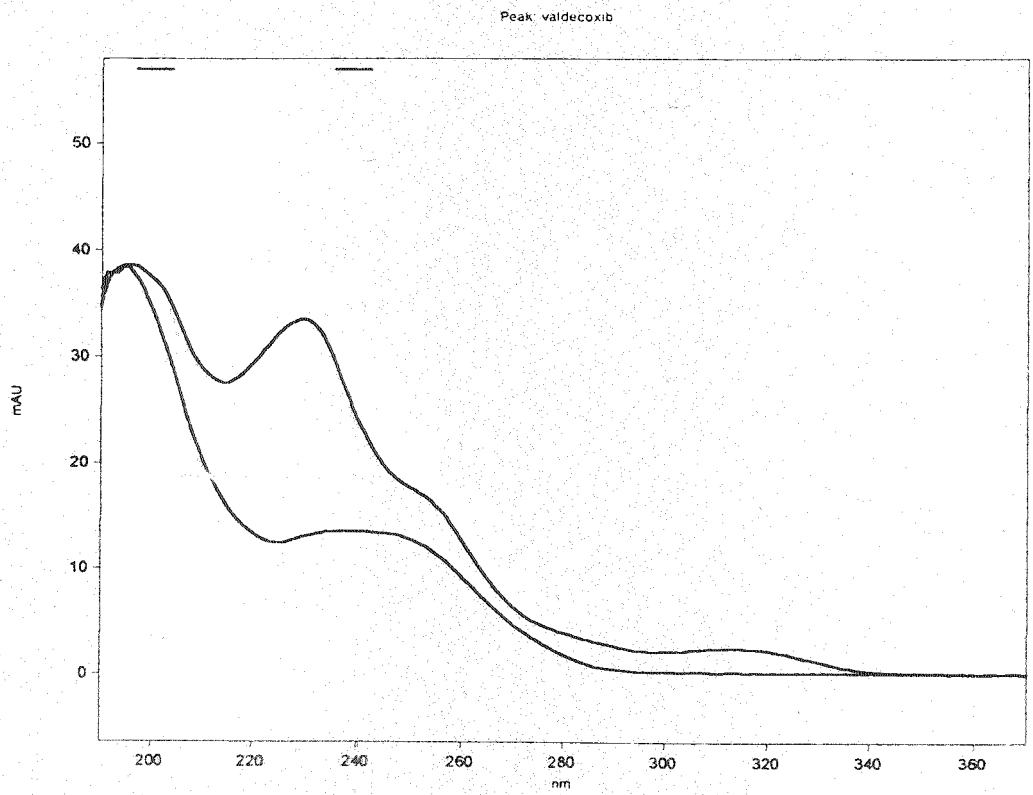

Fig. 2. Overlain UV spectrum of tizanidine and valdecoxib

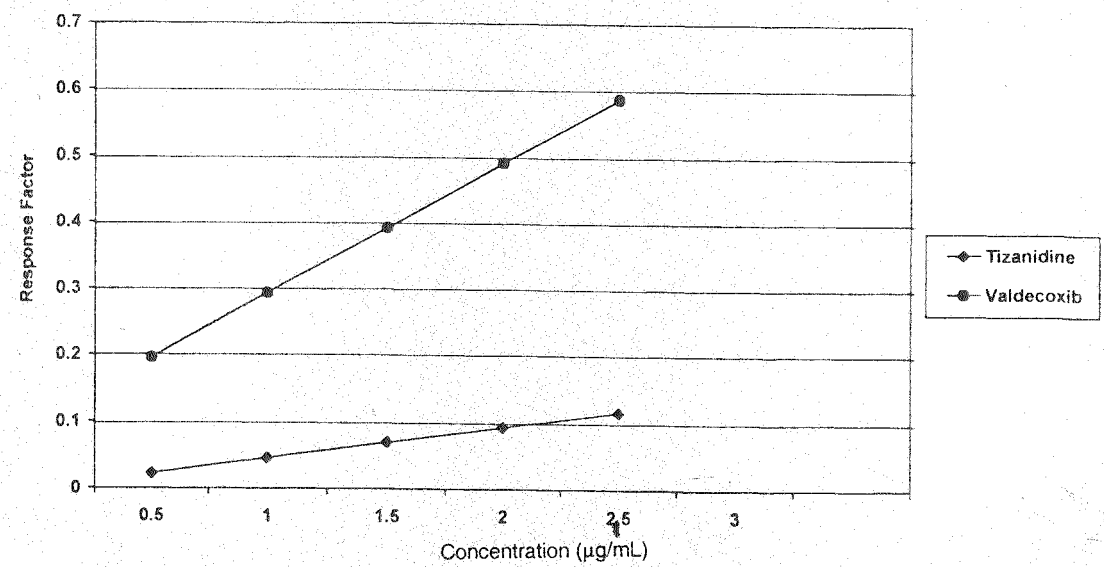

Fig. 3. Caibration curve for tizandine and valdecoxib

making slight changes in the chromatographic conditions. Further there is no interference due to excipients. The system suitability studies were also carried out to determine column efficiency, resolution and peak asymmetry (Table-3). The proposed HPLC method is simple, selective, precise, robust, rugged, linear

and rapid. Hence this method can be applied for the quality control of raw materials, formulations and dissolution studies.

TABLE-3
SYSTEM SUITABILITY STUDIES

S.No.	Parameters	Tizanidine	Valdecoxib
1.	Theoretical plates/meter	25489	29687
2.	Resolution factor	1.70	
3.	Asymmetry factor	1.01	1.04
4.	LOD (ng/mL)	10	5
5.	LOQ (ng/mL)	80	15

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