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Simultaneous Estimation of Rofecoxib and Tizanidine Hydrochloride in Tablets by RP-HPLC

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A simple, precise, accurate and rapid RP-HPLC method has been developed and validated for the simultaneous estimation of rofecoxib and tizanidine hydrochloride in pharmaceutical dosage forms. The process was carried out on C_{18} column (250 × 4.6 mm ID, particle size 5 μm) using a mobile phase of phosphate buffer pH 3.0, acetonitrile and methanol in the ratio of 60:30:10 v/v/v at a flow rate of 1.0 mL/min. The detection of rofecoxib and tizanidine hydrochloride was carried out at a wave length of 274 nm. The calibration curves were linear over the range of 50-250 μg/mL of rofecoxib and 4-20 μg/mL of tizanidine hydrochloride. The retention time of rofecoxib and tizanidine hydrochloride was found to be 4.69 and 2.41 min, respectively. Results of the analysis were validated statistically by recovery studies. The proposed method is simple, rapid and sensitive. It can be successfully used to estimate the drug contents of marketed formulations.

Key Words: Rofecoxib, Tizanidine, RP-HPLC.

INTRODUCTION

Rofecoxib[1] chemically, 4-[4-(methylsulfonyl)-phenyl]-3-phenyl-2(5*H*)-furanone, is a non-steroidal antiinflammatory agent that exhibits antiinflammatory, analgesic and antipyretic activities. It selectively inhibits cyclo-oxygenase II isoenzyme in a dose dependent manner. Tizanidine hydrochloride[2] chemically, 5-chloro-4-(2-imidazolin-2-ylamino)-2,1,3-benzothiadiazole hydrochloride, is a short acting drug for management of spasticity. It is a α_2-adrenergic agonist and centrally acting skeletal muscle relaxant. It has been found to be useful in relieving spasms. Literature survey reveals that various spectrophotometric[3,4] and HPLC[5-12] methods were reported for the individual determination of rofecoxib and tizanidine hydrochloride in pharmaceutical dosage forms. No method has been developed for the estimation of these drugs simultaneously. The present work describes a simple, precise and accurate HPLC method for the simultaneous estimation of rofecoxib and tizanidine hydrochloride in tablet dosage form.

EXPERIMENTAL

Quantitative HPLC was performed on Shimadzu HPLC system consisting of LC-20AT pump, SPD 20A UV-Visible absorbance detector, Shimadzu Spin Chrome software with hypersil ODS C_{18} column (250 × 4.6 mm I.D.; particle size 5 μm). Sample injection was performed *via* a Rheodyne syringe.

Pure samples of rofecoxib and tizanidine hydrochloride were obtained from M/s Aristo Pharmaceuticals Ltd., Mumbai, India and M/s. Sun Pharma Ltd., Baroda, India. The internal standard atorvastatin calcium was obtained from M/s. Micro Labs Ltd., Bangalore, India. Acetonitrile and methanol of HPLC grade were obtained from M/s. Merck Ltd., Mumbai, India. All other chemicals used were of AR grade and obtained from M/s. SD Fine Chemicals Ltd., Mumbai, India.

Chromatographic conditions: The mobile phase used in this study was a mixture of phosphate buffer pH 3.0, acetonitrile and methanol in the ratio of 60:30:10 v/v/v. The run time and the flow rates were 12 min and 1 mL/min, respectively. The mobile phase was filtered before use through 0.45 µ membrane filter and degassed for 15 min. The eluents were monitored at 274 nm. The injection volume was 20 µL.

Preparation of stock solution: Standard solution of the pure drug was prepared by dissolving accurately each 100 mg of rofecoxib and tizanidine hydrochloride in a 100 mL volumetric flask using 25 mL of diluents, acetonitrile:water: methanol in the ratio of 35:30:35 v/v/v. Then the volume made upto the mark with the same solvent and obtains the concentration of 1 mg/mL. Appropriate volume from this stock solution was further diluted to get different concentration levels according to the requirement. Also an accurately 100 mg quantity of atorvastatin calcium was dissolved in diluent to obtain a concentration of 1 mg/mL.

Assay: This method was applied to determine rofecoxib and tizanidine hydrochloride in three different market samples. For analysis of tablet formulation, 20 tablets were weighed and average weight was determined and these were powdered. Sample solution was prepared by dissolving powdered tablets equivalent to 25 mg of rofecoxib and 2 mg of tizanidine hydrochloride in 50 mL volumetric flask. Then the drugs were dissolved by using 25 mL of diluent and the volume was made up to the mark with diluent. The solution was filtered through Whatmann filter paper No. 41 and further diluted with diluent to get a final concentrations of 50, 100, 150, 200 and 250 µg/mL of rofecoxib and 4, 8, 12, 16 and 20 µg/mL of tizanidine hydrochloride. 20 µL of the standard and sample solution were injected, respectively into HPLC system under chromatographic conditions and the chromatograms were recorded. The amount of drug present in tablet formulation was calculated by comparing the mean peak area ratio from the standard. The results are given in Table-1.

TABLE-1
ASSAY OF COMBINED TABLET DOSAGE FORM

Drug	Sample No.	Label claim (mg/tablet)	Amount estimated* (mg/tablet)	Label claim (%)
Rofecoxib	1	25	25.032	100.13
	2	25	25.080	100.32
	3	25	25.135	100.54
Tizanidine	1	2	1.992	99.62
	2	2	1.989	99.48
	3	2	1.996	99.85

*Mean of six determinations.

Method validation: The method was validated interms of linearity, accuracy, intra-day and inter-day precision, reproducibility, specificity, limit of detection (LOD) and limit of quantification (LOQ). Linearity was determined on standard solution by analyzing different concentrations and the calibration curve was plotted. Accuracy of the method was ascertained by recovery studies by adding a known quantity of standard drug to the pre-analyzed sample and the contents were analyzed by the proposed method. The intra-day and inter-day precision was determined by analyzing on the same day and on three different days over a period of two weeks. The intra-day and inter-day variation in the peak area ratio of the drug solution to that of internal standard was calculated in terms of percentage relative standard deviation and the results are shown in Table-2. Specificity was carried out by injecting placebo solution. Robustness of the method was evaluated by performing the assay with variations in wavelength, pH and flow rate. The chromatographic parameters were validated by system suitability parameters and the values are given in Table-3.

TABLE-2
INTRA- AND INTER-DAY PRECISION STUDIES

Drug	Concentration (μg/mL)	Intra-day		Inter-day	
		Mean*	RSD (%)	Mean*	RSD (%)
Rofecoxib	100	100.12	0.262	99.88	0.302
	150	150.70	0.341	150.55	0.301
	200	200.86	0.349	200.63	0.304
Tizanidine	8	8.97	1.512	7.93	1.653
	12	11.90	2.247	11.78	1.990
	16	15.96	2.321	15.82	1.684

*Mean of five determinations.

TABLE-3
SYSTEM SUITABILITY PARAMETERS

Parameter	Rofecoxib	Tizanidine
Linearity (μg/mL)	50-250	4-20
Slope	0.014	0.001
Correlation coefficient	0.999	0.999
Resolution	6.371	6.371
Theoretical plates (N)	8669	2977
Tailing factor	1.00	2.42
Percentage recovery	98.70	99.26
% RSD	0.284	0.175
LOD (μg/mL)	0.240	0.018
LOQ (μg/mL)	0.801	0.063

RESULTS AND DISCUSSION

The retention time for rofecoxib and tizanidine hydrochloride was found to be 4.69 and 2.41 min, respectively. A typical chromatogram of rofecoxib and tizanidine hydrochloride is shown in Fig. 1. Linearity was observed in the concentration range

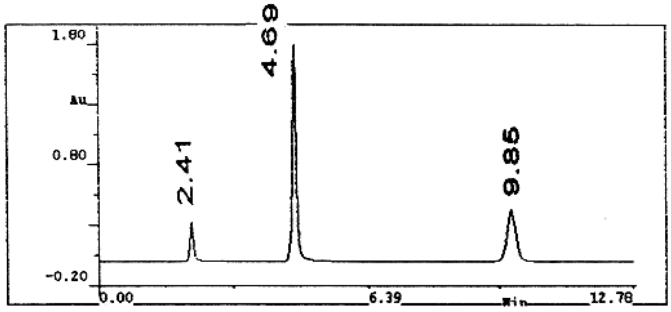

Fig. 1. Typical chromatogram of rofecoxib and tizanidine hydrochloride

of 50-250 µg/mL for rofecoxib and 4-20 µg/mL for tizanidine hydrochloride, with correlation coefficient of 0.999 for rofecoxib and 0.999 for tizanidine hydrochloride, respectively. The high percentages of recovery of the drugs indicate that the method is highly accurate. Recovery data from the study is reported in Table-4. There was good repeatability of proposed method with high percentage RSD 0.284 for rofecoxib and 0.175 for tizanidine hydrochloride. No interfering peaks were found in the chromatogram indicating that the excipients in tablet formulations did not interfere with the estimation of the drug and the peak response was due to individual drug components only. The LOD and LOQ for rofecoxib were found to be 0.240 and 0.801. The LOD and LOQ for tizanidine hydrochloride were found to be 0.018 and 0.063.

TABLE-4
RECOVERY STUDIES

Formulation	Drug	Label claim (mg)	Amount added (mg)	Amount recovered (mg)	Recovery (%)
Brand-1	Rofecoxib	25	50	49.49	98.78
	Tizanidine	2	4	3.99	99.75
Brand-2	Rofecoxib	25	100	98.71	98.71
	Tizanidine	2	8	7.95	99.37
Brand-3	Rofecoxib	25	150	147.90	98.60
	Tizanidine	2	12	11.84	98.66

The proposed HPLC method was found to be simple, rapid, specific, precise and accurate for the estimation of rofecoxib and tizanidine hydrochloride in tablet dosage forms. Hence, it can be easily and conveniently adopted for routine quality control analysis.

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