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DEVELOPMENT AND VALIDATION OF A HPTLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ROFECOXIB AND TIZANIDINE HYDROCHLORIDE IN TABLET DOSAGE FORM

A. LAKSHMANA RAO* and D. PRAVEEN VARMA

*Department of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy, Vishnupur, BHIMAVARAM - 534 202 (A. P.) INDIA

ABSTRACT

A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of rofecoxib and tizanidine hydrochloride simultaneously in combined pharmaceutical dosage forms. The stationary phase used was pre-coated silica gel $60F_{254}$. The mobile phase used was a mixture of methanol : acetone in the ratio of 1 : 1v/v. The detection of spots was carried out at 254 nm. The method was validated in terms of linearity, accuracy, precision, specificity and reproducibility. The calibration curve was found to be linear between 2.204 to 3.016 µg/spot for rofecoxib and 0.180 to 0.260 µg/spot for tizanidine hydrochloride. The limit of detection and limit of quantification for rofecoxib were found to be 0.70 and 2.20 µg/spot, respectively and for tizanidine hydrochloride 0.052 and 0.16 µg/spot, respectively. The proposed method can be successfully used to determine the drug content of marketed formulation.

Key words : Rofecoxib, Tizanidine, HPTLC.

INTRODUCTION

Rofecoxib[1] chemically, 4-[4-(methylsulfonyl)-phenyl]-3-phenyl-2(5H)-furanone, is a non-steroidal anti-inflammatory agent that exhibits anti-inflammatory, 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. Rofecoxib and tizanidine hydrochloride are available in combined tablet dosage form, where both the drugs act synergistically in

* Author for correspondence; Email : dralrao@gmail.com

relieving the muscle spasms. Various analytical methods like spectrophotometry[3,4] and HPLC[5-12] methods have been reported for the individual determination of rofecoxib and tizanidine hydrochloride in pharmaceutical dosage forms. No HPTLC method has been reported for the estimation of rofecoxib and tizanidine hydrochloride in combined dosage forms so far. In the present investigation, an attempt has been made to develop a simple, precise and accurate HPTLC method for the simultaneous estimation of rofecoxib and tizanidine hydrochloride in combined dosage form.

EXPERIMENTAL

Materials and methods

Rofecoxib and tizanidine hydrochloride were procured as gift samples from Aristo Pharmaceuticals Ltd., Mumbai and Sun Pharmaceuticals Ltd., Baroda, respectively. The pre-coated silica gel $60F_{254}$ TLC plates (20 x 20 cm, layer thickness 0.2 mm) obtained from E. Merck, Mumbai were used as a stationary phase. The mobile phase used was methanol : acetone (1 : 1v/v). All the solvents used were of AR grade, obtained from S. D. Fine Chemicals Ltd., Mumbai. The combined tablet formulation contains 25 mg of rofecoxib and 2 mg of tizanidine hydrochloride were purchased from a local pharmacy. A Camag HPTLC System comprising of Camag Linomat V semi automatic sample applicator, Camag TLC scanner, Camag twin trough chamber, Camag CATS software, Hamilton syringe (100 µL) and ultrasonicator were used during study.

Preparation of standard and sample solutions

A standard solution of rofecoxib and tizanidine hydrochloride was prepared with accurately weighed quantities of rofecoxib (100 mg) and tizanidine hydrochloride (100 mg), into a 100 mL volumetric flask. The content was dissolved in methanol and the volume made up to 100 mL with methanol and obtains the concentration of 1 mg/mL of each drug. Twenty tablets were weighed accurately and ground to fine powder. The quantity of powder equivalent to 25 mg of rofecoxib and 2 mg of tizanidine hydrochloride were transferred to a conical flask and dissolved in methanol. The solution was sonicated for
15 min. The extract was filtered through Whatmann filter paper No. 41 and the residue was washed with methanol. The extract and washings were transferred into 50 mL volumetric flask and the volume was made up to 50 mL with methanol. Required dilutions were made to obtain suitable concentrations of rofecoxib and tizanidine hydrochloride.

Chromatographic conditions

The TLC plates were pre-washed with methanol. Activation of the plates was done in an oven at 50°C for 5 min. The chromatographic conditions maintained were pre-coated silica gel $60F_{254}$ aluminium sheets (20 x 20 cm) as stationary phase, methanol : acetone (1 : 1v/v) as mobile phase, chamber and plate saturation time of 30 min., migration distance allowed was 72 mm, wave length scanning was done at 254 nm keeping the slit dimension at 5 x 0.45 mm, scanning speed 20 nm/sec, the source of radiation was a deuterium lamp.

Calibration curve

Three microlitres of standard stock solution of rofecoxib and tizanidine hydrochloride and aliquot of formulation were spotted on pre-coated TLC plates with the help of Linomat V semi automatic sample applicator. The plate was developed with 20 x 10 twin trough chamber up to 82 mm. The plate was dried in air after development, scanned with Camag TLC scanner using CATS software incorporating the track optimization option. The calibration curves were prepared by plotting peak area versus concentration (μg/spot) corresponding to each spot.

Validation of the method

The developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision, specificity, limit of detection, limit of quantification and repeatability of measurement of peak, as well as repeatability of sample application.

Analysis of the marketed formulations

Three microlitres of the sample solutions of the marketed formulation was spotted on to the sample plate followed by development scanning.

Table 1 : Results of analysis of marketed formulation

Formulation	Label claim (mg/tablet)	Amount found* (mg)	% of drug found*	% RSD
Rofecoxib	25	24.89	99.56	0.263
Tizanidine	2	2.047	102.35	0.449

*Mean of six determinations.

The analysis was repeated six times. The spots were resolved into two peaks in the chromatogram of drug samples extracted from the marketed formulations. The content of

the drug was calculated from the peak areas recorded. The amount obtained and result of analysis shown in Table 1.

Tablet contains 25 mg of rofecoxib and 2 mg of tizanidine hydrochloride.

RESULTS AND DISCUSSION

A solvent system that would give dense and compact spots with appropriate and significant R_f values was desired for quantification of rofecoxib and tizanidine hydrochloride in pharmaceutical formulations. The mobile phase consisting of methanol : acetone (1 : 1v/v) gave R_f values of 0.67±0.04 and 0.45±0.04 for rofecoxib and tizanidine hydrochloride, respectively. The typical chromatogram of rofecoxib and tizanidine hydrochloride after separation is shown in Fig. 1.

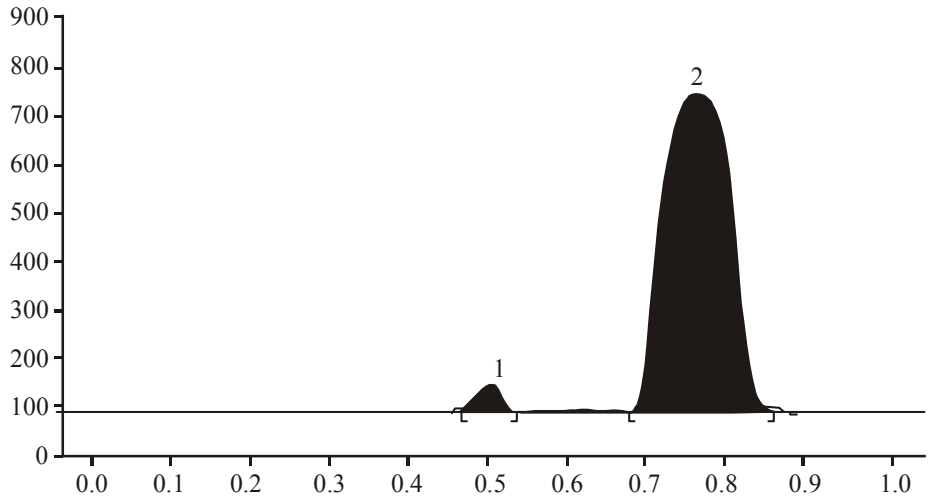

Fig. 1: Chromatogram of rofecoxib and tizanidive

Linearity range for rofecoxib and tizanidine hydrochloride was found to be in the range of 2.204 to 3.016µg/spot and 0.180 to 0.260µg/spot, with a correlation coefficient of 0.999 and 0.999, respectively. The limit of detection and limit of quantification for rofecoxib were found to be 0.70 and 2.20µg/spot, respectively and for tizanidine hydrochloride were found to be 0.052 and 0.16µg/spot, respectively. The intra-day precision was determined by analyzing standard solutions in the concentration range of 2.632 to 3.016µg/spot for rofecoxib and 0.220 to 0.260µg/spot for tizanidine hydrochloride for three times on the same day while inter-day precision was determined by analyzing corresponding standards daily for three days over a period of one week. The intra-day and inter-day coefficients of variation for both the drugs indicated that the method is precise

and the values are given in Table 2.

Table 2 : Precision data of rofecoxib and tizanidine hydrochloride

Drug	Concentration (µg/spot)	Intra-day precision % RSD	Inter-day precision % RSD
Rofecoxib (25 mg)	2.632	99.85 ± 0.75	100.61 ± 0.43
	2.820	100.30 ± 1.49	101.52 ± 0.74
	3.016	100.75 ± 0.69	101.41 ± 0.84
Tizanidine (2 mg)	0.220	101.24 ± 1.29	102.12 ± 0.52
	0.244	102.10 ± 0.38	101.64 ± 0.27
	0.260	101.20 ± 1.84	102.04 ± 0.79

RSD-Relative standard deviation.

Repeatability of sample application was assessed by spotting 2 µL of drug solution five times on a TLC plate followed by development of plate and recording the peak area for 5 spots. The % RSD for peak area values of rofecoxib and tizanidine hydrochloride was found to be 1.41 and 2.51, respectively. Repeatability of measurement of peak area was determined by spotting 2 µL of rofecoxib and tizanidine hydrochloride solution on a TLC plate and developing the plate.

Table 3 : Recovery studies of rofecoxib and tizanidine hydrochloride

Formulation	Drug	Label claim (mg/tablet)	Amount added (mg)	Amount recovered* (mg) ± SD	% Recovery ± SD
Brand-1	Rofecoxib	25	4.212	3.962	102.42±0.65
	Tizanidine	2	2.084	2.141	99.44±0.82
Brand-2	Rofecoxib	25	4.292	4.042	102.68±0.43
	Tizanidine	2	2.132	2.141	99.89±0.71

*Each value is a mean± standard deviation of three determinations.

The separated spot was scanned five times without changing the position of the plate and % RSD of measurement of peak area for rofecoxib and tizanidine hydrochloride were found to be 0.65 and 0.18, respectively. To confirm the specificity of the proposed method, the solution of the formulation was spotted on the TLC plate, developed and

scanned. It was observed that the excipients present in the formulation did not interfere with the peaks of rofecoxib and tizanidine hydrochloride. Recovery studies of the drugs were carried out for the accuracy parameter. These studies were carried out at three levels i. e., multiple level recovery studies. Sample stock solutions from tablet formulation of 2.204µg/mL for rofecoxib and 0.180 µg/mL for tizanidine hydrochloride were prepared. Dilutions were made and recovery studies were performed. Percentage recovery was found to be with in the limits as listed in Table 3.

The assay value of rofecoxib and tizanidine hydrochloride for the marketed formulation was found to be with in the limits. The low RSD value indicated the suitability of the method for routine analysis of rofecoxib and tizanidine hydrochloride in pharmaceutical dosage forms. Different validation parameters for the proposed HPTLC method for determining rofecoxib and tizanidine hydrochloride were summarized in Table 4.

Table 4 : Validation parameters

Parameter	Value	
	Rofecoxib	**Tizanidine**
Linearity (µg/spot)	2.204-3.016	0.180-0.260
Correlation coefficient (r)	0.999	0.999
LOD (µg/spot)	0.70	0.052
LOQ (µg/spot)	2.20	0.16
Precision (% CV)	2.632-3.016	0.220-0.260
Repeatability of application (n = 5)	1.41	2.51
Repeatability of measurement (n = 5)	0.65	0.18
Specificity	specific	Specific
% recovery	102.55	99.66
Accuracy (% RSD)	0.263	0.449

CONCLUSION

The developed HPTLC technique is simple, rapid, precise, specific and accurate and the statistical analysis proved that the method is reproducible and selective for the estimation of rofecoxib and tizanidine hydrochloride simultaneously in bulk drug and

tablet formulations.

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