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Validated High Performance Thin Layer Chromatography Method for Simultaneous Estimation of Rofecoxib and Tizanidine Hydrochloride in Pure and Tablet Dosage Forms

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A simple, specific, precise and rapid HPTLC method has been developed for estimation of rofecoxib and tizanidine hydrochloride simultaneously in pure and tablet dosage form. In this method standard solution and sample solution were applied on a pre coated silica gel 60 F_{254} TLC plate and developed using a mixture of acetone: methanol (1:1 v/v). The method was validated in terms of linearity, accuracy, precision, repeatability and specificity proving that this method is effective for the simultaneous estimation of the drug content in pure and tablet dosage form.

Key Words: HPTLC, Rofecoxib, Tizanidine Hydrochloride.

INTRODUCTION

Rofecoxib (ROF) chemically, 4-[4-(methyl sulfonyl)-phenyl]-3-phenyl-2-(5H)-furone is a selective cyclooxygenase-2 (cox-2) inhibitor that exhibit antiinflammatory, analgesic and antipyretic activities[1,2]. Tizanidine hydrochloride (TIZ) chemically, 5-chloro-N-(4,5-dihydro-1H-imidazol-2-yl)-2,1,3-benzothiazol-4-amine hydrochloride[3]., is a agonist at α-2-adrenergic receptor site and exhibit relief in skeletal muscle spasm[1,4]. Various methods are available in literature like HPLC[5-8], LC-MS[9], UV and visible spectrophotometry[10,11] for ROF. GC-MS[12], RP-HPLC[13,14], extractive spectrophotometric methods[15] for TIZ. These are the methods applied for the estimation of ROF and TIZ in pharmaceutical preparation or biological fluids.

There is no reported HPTLC method for the estimation of these drugs simultaneously in bulk and pharmaceutical preparation. The present study describes the development and validation of simple, specific, sensitive accurate and precise HPTLC method for the estimation of ROF and TIZ simultaneously in tablet dosage form.

EXPERIMENTAL

Rofecoxib and tizanidine hydrochloride in pure form were obtained as gift samples from Aristo Pharma, Mumbai. Silica Gel 60 F_{254} TLC plates (20 × 20 cm, layer thickness 0.2 mm, E. Merk, Germany) were used as the stationary phase. 20 Tablets [ROF (25 mg) and TIZ (2 mg)] were purchased from the local pharmacy. Methanol and acetone of AR grade purity were procured from E.Merk Ltd, Mumbai. Camag automatic TLC sample applicator III, Camag TLC scanner II with CATS evaluation software (version 4.0) were used in the studies (Camag, Mutteg, Switzerland).

Standard and sample preparation: Working standard of ROF and TIZ (100 mg each) were weighed accurately and diluted with mobile phase [methanol:acetone (1:1 v/v)] to obtain the final concentration of 100 µg/mL. 20 Tablets were separately crushed and grounded to a fine powder. A weight equivalent to 25 mg of ROF and 2 mg of TIZ were transferred into a 10 mL standard flask. The contents were dissolved in mobile phase and volume was made up to the mark. The contents were mixed well using ultrasonicator and filtered through whatmann filter paper. The filtered solution was then used for the estimation.

HPTLC method and chromatographic condition: TLC plates were pre-washed with methanol and dried in hot air. The chromatographic condition maintained were precoated Silica Gel 60 F_{254} aluminium sheet (10 × 10 cm) as stationary phase, methanol:acetone (1:1v/v) as mobile phase, migration distance was allowed up to 80 mm. Wavelength scanning was done at 254 nm. Keeping slit dimension of 5.0 × 0.45 mm. A deuterium lamp provided the source of radiation. 10 µL of standard solution (100 µg/mL) of each drug were applied in the pre-washed TLC plates. It was then developed in a Camag twin-trough chamber previously saturated for 0.5 h with 10 mL of mobile phase.

The plates were removed from the chamber and dried in air. Densitometry measurements were performed at 254 nm with Canmag scanner III using CATS 4 software incorporating the tract optimization option. For plotting the calibration curve aliquots 2, 2.2, 2.4, 2.6, 2.8 and 3 µg were prepared from the standard solution of ROF (100 µg/mL) and 0.18, 0.20, 0.22, 0.24 and 0.26 µg were prepared from the standard solution of TIZ (100 µg/mL) were applied on the TLC plates using automatic sample applicator III under nitrogen stream. The TLC plates were dried, developed and densitometrically analyzed as described earlier.

Assay of tablet formulation: Sample solution was spotted with volume 5 and 10 µL on to the TLC plate followed by developing and scanning. The analysis was repeated in triplicate. The spot was resolved into three peaks in the chromatogram of drugs samples, extracted from the tablet formulation. The content of the drug was calculated from the peak area.

Method validation: The method was validated as per ICH guidelines in terms of linearity, accuracy, inter-day, intra-day, precision, reproducibility of measurement of peak area, reproducibility of sample application and specificity. The limit of quantification and limit of detection of ROF and TIZ were determined. Accuracy of the analysis was carried by recovery studies. For those studies, known concentration of the drug is added to preanalyzed tablet and recovery was calculated.

The intra-day precision were determined by analyzing standard drug solution in the concentration range from 500 to 1000 ng/spot for thrice on the same day, while inter-day precision were determined by analyzing corresponding standard daily for a period of 1 week.

Repeatability of measurement of peak area was determined by spotting 10 µL of standard drug solution on a TLC plate developed and analyzed. The separated spot were scanned 7 times without changing the position of the plate and relative standard deviation (RSD) for measurement of the peak were calculated. Repeatability of sample application was assessed by spotting 10 µL of standard drug solution 7 times on TLC plate by automatic applicator followed by development of the plate and recording the peak area for 7 spots. The RSD for the peak were calculated.

The specificity of the proposed method was checked by spotting a sample of ROF and TIZ on the TLC plate, developed and scanned. Purity was checked by overlaying the spectra of sample recorded on a TLC scanner in UV range.

RESULTS AND DISCUSSION

The developed HPTLC method was found to be easy and cost effective when considered with that of HPLC or LC-MS for routine analysis. The solvent system having a combination of methanol:acetone (1:1 v/v) offered a maximum resolution for the two drugs. R_f value of ROF 0.68 ± 0.03 and R_f value of TIZ is 0.48 ± 0.03. After development, the plates were scanned at wavelength 254 nm. Since the drugs are freely soluble in mobile phase, the tablet powder was extracted with mobile phase, sonicated for 10 min, which helped to extract them completely from tablet matrix.

The amount of drug in tablet formulation was calculated on applying the dilution factor and comparing the peak area of the standard and sample solution. The assay of ROF and TIZ in tablet formulation calculated as per peak area was found to be 99.6 ± 0.03 and 101.95 ± 0.05 (Table-1). The good average recovery values obtained in recovery studies indicate that the proposed method is accurate for the estimation of drug in tablet (Table-2).

TABLE-1
ASSAY OF ROFECOXIB (ROF) AND TIZANIDINE HYDROCHLORIDE (TIZ)

Labeled claim	Amount found ± SD* (mg)	Assay ± SD* (%)	CV (%)
ROF -25 mg	24.95 ± 0.6557	99.8 ± 0.6500	0.2634
TIZ -2 mg	2.03 ± 0.0019	101.9 ± 0.0019	0.4490

*Average value ± standard deviation of five determinations.

TABLE-2
RECOVERY OF ROFECOXIB AND TIZANIDINE HYDROCHLORIDE

Label claim (mg/table)	Amount added (mg)	Amount of drug present in pre analytical sample (mg)	Amount recovered* (mg)	Recovery* (%)	Average recovery
ROF (25 mg)	4.212	28.962	29.665 ± 0.65	102.42 ± 0.63	102.55 ± 0.620
	4.292	29.042	29.823 ± 0.62	102.68 ± 0.61	
TIZ (2 mg)	2.084	4.225	4.2017 ± 0.019	99.44 ± 0.022	99.65 ± 0.020
	2.132	4.273	4.2684 ± 0.016	99.89 ± 0.019	

*Average value ± standard deviation of five determinations.

The intra-day and inter-day coefficient of the drug were found to be in the range 0.26-2.3 and 0.3-1.9 %, respectively. Lower values of intra-day and inter-day variation in the analysis indicate that the method is precise. The RSD for repeatability of measurement of peak area and RSD for repeatability of sample application was found well below the instrumentation specification ensuring proper function of HPTLC system.

It was observed that the excipients present in the formulation did not interfere with peak of ROF ($R_f = 0.67 \pm 0.03$) and TIZ ($R_f = 0.45 \pm 0.03$). The purity was confirmed by overlaying the spectra of standard mixture of drugs with spectra of sample recorded on TLC scanner in UV range, which shows the specificity of method. Different parameter for validation of the proposed method were summarized in Table-3.

The proposed HPTLC method was found to be rapid, cheaper, simple, specific, sensitive, precise and accurate. Thus it can be applied for the routine quality control analysis of ROF and TIZ for pure and tablet dosage forms.

TABLE-3
METHOD VALIDATION PARAMETERS

Parameter	Results	
	ROF	TIZ
Linearity range (μg)	2.2 -3.2	0.18 - 0.26
Correlation coefficient (r)	0.999	0.999
Limit of detection (LOD)	0.240	0.018
Limit of quantification (LOQ)	0.801	0.063
Accuracy	102.55	99.66
Precision (% CV)		
Repeatability of application (n = 7)	0.78	0.76
Repeatability of application (n = 7)	0.26	0.44
Intra-day (n = 3)	0.260-0.349	1.50-2.30
Inter-day (n = 3)	0.302-0.304	1.65-1.99
Specificity	Specific	Specific

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