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Short communication

Simultaneous LC determination of tizanidine and rofecoxib in tablets

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Abstract

A reverse phase high performance liquid chromatographic method to determine tizanidine (TZ) and rofecoxib (RF) in combination is proposed and applied to the pharmaceuticals. This method allows the determination of 0.1–0.5 μg/ml of TZ and 1.2–6.0 μg/ml of RF along with 10 μg/ml of nimesulide (internal standard), in a mobile phase consisting of 1% (v/v) triethylamine (pH adjusted to 2.5 using dilute orthophosphoric acid):acetonitrile in the ratio 55:45% (v/v). Detection wavelength of 303 nm and flow rate of 0.8 ml/min were fixed for the study. The limit of detection (LOD) for TZ and RF were found to be 10 and 1 ng/ml, respectively. The limit of quantification (LOQ) for TZ and RF were found to be 80 and 12 ng/ml, respectively. The amount of drug present in the tablet and the recovery studies were also carried out. The % R.S.D. of recovery studies for TZ and RF were found to be 0.0673 and 0.0146, respectively. The method is validated for accuracy, precision, ruggedness and robustness.

Keywords: Tizanidine; Rofecoxib; Simultaneous HPLC

1. Introduction

Tizanidine hydrochloride (5-chloro-4-(2-imidazolin-2-yl amino)-2,1,3-benzothiodiazole hydrochloride) (TZ) is a short acting drug for management of spasticity [1]. It is an agonist at α_2-adrenergic receptor sites. Two and 4 mg tablets are available for oral administration. Rofecoxib (4-(4-(methyl sulfonyl) phenyl)-3-phenyl-2 (5H)-furanone) (RF) is a non-steroidal anti-inflammatory drug that exhibits anti-inflammatory, analgesic, and antipyretic activities [2]. 12.5, 25 and 50 mg tablets are available for oral administration. RF and TZ are available in combined tablet dosage forms, each contains 25 mg of RF and 2 mg of TZ.

Spasticity is a common and disabling symptom for patients with motor neuron dysfunction. Pain is associated with various conditions of spasticity. TZ is a centrally acting skeletal muscle relaxant, which has been found to be useful in relieving spasms, and RF is a selective cox-2 inhibitor found to be useful in the management of pain and inflammation associated with various conditions.

So far very few liquid chromatography procedures have been described for the determination of TZ and RF [3–17]. These procedures were developed to estimate either TZ or RF individually, from formulation or plasma. Whereas no single method has been reported for their simultaneous estimation. Hence, it is necessary to develop a rapid, accurate and validated method for the determination of TZ and RF from combined dosage form.

2. Experimental

2.1. Apparatus

The HPLC system was Shimadzu class LC-10A (Japan), including pump LC-10AT, SPD-10A UV–vis detector and a communication bus module CBM-10A. Waters, spherisorb ODS column (150 mm × 4.6 mm i.d. 0.5 μm) was used as the stationary phase. The pH meter used was Elico Pvt. Ltd. India.

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2.2. Reagents and chemicals

TZ and RF were received as gift samples from Unichem Pharmaceuticals Ltd., Roha and Reddys Pharmaceuticals Ltd., Hyderabad, India, respectively. Triethylamine LR grade, orthophosphoric acid LR grade, acetonitrile for HPLC and water for HPLC were purchased from S.D. Fine chemicals Limited, India and Qualigens fine Chemicals, India.

2.3. Standard solutions

A stock solution containing 100 μg/ml of TZ and 1200 μg/ml RF were prepared by dissolving TZ and RF in acetonitrile. A working standard solution containing 10 μg/ml TZ and 120 μg/ml of RF were prepared from the above stock solution. A stock solution of nimesulide (1000 μg/ml) was prepared and used as internal standard. All the solutions were covered with aluminium foil to prevent photolytic reaction until the time of analysis.

2.4. HPLC conditions

The mobile phase used was 1% (v/v) triethylamine:acetonitrile in the ratio 55:45% (v/v). Prior to use, the mobile phase was mixed thoroughly and degassed. The mobile phase was pumped at 0.8 ml/min. The eluents were monitored at 303 nm. The injection volumes for samples and standards were 20 μl.

2.5. Analysis of formulation

Twenty tablets, each containing 2 mg of TZ and 25 mg of RF were weighed, average weight calculated. A quantity of powder equivalent to 10 mg of TZ and 120 mg of RF was weighed accurately and transferred to a 100 ml standard flask. The internal standard (nimesulide) was added to standard flask. The drug was initially dissolved in acetonitrile and 0.1N HCl and the volume made up with mobile phase. Each solution contained 10 μg/ ml of nimesulide. The solution was filtered using 0.2 μm membrane filter. The aliquot was then suitably diluted to the linearity range. Then 20 μl of these solutions was injected in to the column and chromatogram was recorded and shown in Fig. 1. The retention times of TZ, RF and nimesulide were found to be 1.6, 3.2, 5.3 min, respectively (Fig. 1).

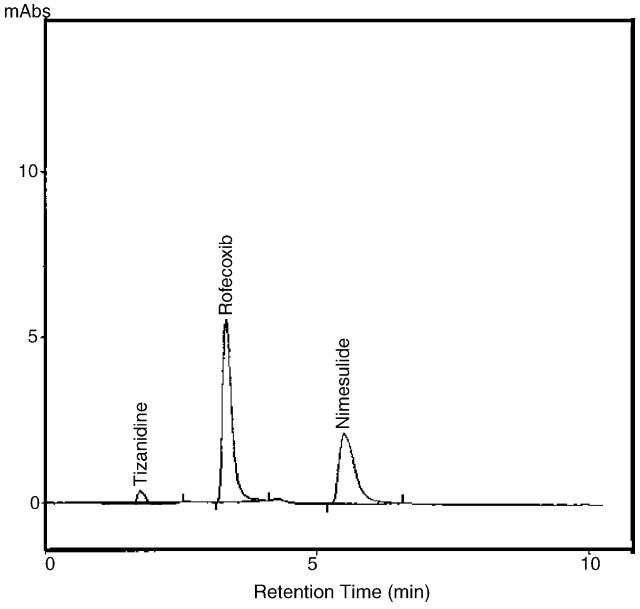

Fig. 1. Chromatogram of TZ and RF and internal standard.

3. Results and discussion

A series of standard solutions (three replicates of each) of TZ and RF in combination were measured by following the procedure described in Section 2. A calibration graph was plotted using peak area ratio of the standard peak areas to that of internal standard peak area versus concentration of the standard solutions expressed in μg/ml. The calibration graph was found to be linear in the range of 0.1–0.5 μg/ml for TZ and 1.2–6.0 μg/ml for RF. The slope, intercept and correlation values were found to be 1.0048, 0.1398 and 0.9997, respectively, for TZ and 1.2721, −0.0051 and 0.9998, respectively, for RF.

Precision of the method was studied by repeatedly injecting the mixture of TZ and RF. The % R.S.D. was found to be 0.15 and 0.52 for TZ and RF, respectively.

The LOD and LOQ of the developed method were determined by injecting progressively low concentration of the standard solution under optimized chromatographic conditions. The LOD of TZ and RF were found to be 10 and 1 ng/ml, respectively, and the LOQ was found to be 80 and 12 ng/ml for TZ and RF, respectively.

To study the reliability and accuracy of the method, recovery experiments were carried out at 50 and 100% levels. The % R.S.D. of the recovery studies is 0.0673 and 0.0146 for TZ and RF, respectively.

For demonstrating the stability of TZ and RF, they were observed in room temperature and under refrigeration. Any change in the retention time, resolution, tailing of peaks, etc., compared to the pattern of the chromatogram of freshly prepared solution. The solution stored under room temperature was stable up to 4 h and under refrigeration up to 2 days.

A placebo prepared using lactose, carboxy methylcellulose and starch was used to study the effect of excipients on quantification of TZ and RF. There was no interference found due to the excipients was supported by the % R.S.D. values of recovery studies (0.0673 for TZ and 0.0146 for RF).

The system suitability studies were carried out as specified in USP. These parameters include column efficiency (N), resolution (R_s), capacity factor (K'), selectivity factor (α), peak asymmetry factor (A_s), % R.S.D. for peak area or height of repetitive injection, etc. For these calculations, chromatograms of the mixed standard solutions were used and are shown in Table 1.

Table 1
System suitability study

	R_s	N	K'	α	A_s
Tizanidine	2.46	12844	1.1	–	1.03
Rofecoxib	2.47	35560	3.1	2.78	0.98

Table 2
Result of tizanidine and rofecoxib determination in tablets

	Labelled amount (mg/tablet)	Amount found (mg/tablet)[a]	% R.S.D.
Tizanidine	2	2.10 ± 0.10	0.15
Rofecoxib	25	24.21 ± 0.15	0.11

[a] Mean ± S.D. of six observations.

The study was applied for pharmaceutical formulation and the amount obtained and % R.S.D. are shown in Table 2.

The results obtained by this method are precise and reproducible for the two drugs, TZ and RF in combination. The recovery values are satisfactory and show the reliability and accuracy of the method. The validation study shows that the developed method is accurate and robust. Further this method eliminates complicated extraction of individual drugs for quantitation. Both the drugs were estimated within 10 min. Hence the present method is cost effective and faster analytical method. The application of suggested procedure was successfully applied to the detection of these drugs in pharmaceutical preparation with high % recovery, good accuracy and precision. Hence this developed method is simple, sensitive and readily adaptable to routine determination of TZ and RF in combined formulation.

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