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Application of HPLC and HPTLC for the simultaneous determination of tizanidine and rofecoxib in pharmaceutical dosage form

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Abstract

Two methods are described for the simultaneous determination of tizanidine and rofecoxib in binary mixture. The first method was based on HPTLC separation of the two drugs followed by densitometric measurements of their spots at 311 nm. The separation was carried out on Merck HPTLC aluminium sheets of silica gel 60 F_{254} using toluene:methanol:acetone (7.5:2.5:1.0, v/v/v) as mobile phase. The linear regression analysis data was used for the regression line in the range of 10–100 and 100–1500 ng/spot for tizanidine and rofecoxib, respectively. The second method was based on HPLC separation of the two drugs on the reversed phase kromasil column [C_{18} (5 μm, 25 cm × 4.6 mm, i.d.)] at ambient temperature using a mobile phase consisting of phosphate buffer pH 5.5 and methanol (45:55, v/v). Flow rate was 1.0 ml/min with an average operating pressure of 180 kg/cm^2. Quantitation was achieved with UV detection at 235 nm based on peak area with linear calibration curves at concentration ranges 10–200 and 100–2000 μg/ml for tizanidine and rofecoxib, respectively. Both methods have been successively applied to pharmaceutical formulation. No chromatographic interference from the tablet excipients was found. Both methods were validated in terms of precision, robustness, recovery and limits of detection and quantitation. The analysis of variance (ANOVA) and Student's *t*-test were applied to correlate the results of tizanidine and rofecoxib determination in dosage form by means of HPTLC and HPLC method.

Keywords: Tizanidine; Rofecoxib; HPTLC; Reversed phase HPLC; Method validation; Quantitative analysis; ANOVA; Student's *t*-test

1. Introduction

Tizanidine 5-chloro-4-(2-imidazolin-2-ylamino)-2,1,3-benzothiadiazole (Fig. 1) is α_2 – adrenergic agonist and centrally active myotonolytic skeletal muscle relaxant with a chemical structure unrelated to other muscle relaxants [1,2]. It reduces spasticity by increasing presynaptic inhibition of motor neurons. The effects of tizanidine are greatest on polysynaptic pathways. The overall effect of these actions is thought to reduce facilitation of spinal motor neurons. It also reduces increased muscle tone associated with spasticity in patients with multiple sclerosis or spinal cord injury. The plasma concentration of tizanidine after oral administration is presumed to be several nanograms [3]. In the literature, a radioimmunoassay method for the quantification of tizanidine hydrochloride has been widely used [4]. Also determination of tizanidine in human plasma by gas chromatography–mass spectrometry has been reported [5]. Tizanidine, which contains a cyclic guanidine moiety, can exist as two tautomers [6]. There are very few reports on analytical methods for estimation of tizanidine in bulk and its dosage form. A RP–HPLC method for estimation of tizanidine hydrochloride in combination with nimesulide has been reported by Raman and Patil [7]. Qi et al. [8] have reported stability indicating HPLC method for tizanidine. Mahadik et al. [9] have reported stability indicating HPTLC method for tizanidine hydrochloride.

Rofecoxib chemically 4-(4-methanesulfonylphenyl)-3-phenyl-5H-furan-2-one (Fig. 2) is a new generation

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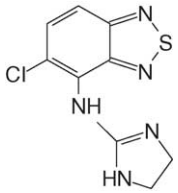

Fig. 1. Structure of tizanidine.

non-steroidal anti-inflammatory agent (NSAID) that exhibits promising anti-inflammatory, analgesic and antipyretic activity. It selectively inhibits cyclo-oxygenase II (COX-2) isoenzyme in a dose dependent manner in man [10–13]. COX-2 is found in elevated levels in inflammatory exudates [14,15]. Rofecoxib (a specific COX-2 inhibitor) selectively targets the prostaglandins involved in pain and inflammation. Several methods for quantitative estimation of rofecoxib in pharmaceutical dosage form and in biological fluids have been reported in the literature. Woolf et al. [16] has reported HPLC method for rofecoxib in plasma with post column photochemical derivitization and fluorescence detection. Matthews et al. [17] have described LC method after solid phase extraction with fluorescence detection. Several LC–MS methods for determination of rofecoxib in human plasma have been reported [18–20]. Simple reverse phase HPLC method for quantitative estimation of rofecoxib in pharmaceutical formulation [21] and from human plasma [22–24] has been reported. Mao et al. [25] has reported stability indicating HPLC method for rofecoxib. Isolation and characterization of process related impurities in rofecoxib have been reported [26].

The present work presents two new methods for simultaneous determination of tizanidine and rofecoxib in tablets using HPTLC-densitometry and reverse phase HPLC. The two methods are simple, reduce the duration of the analysis and suitable for routine determination of two drugs.

2. Experimental

2.1. Materials

Pharmaceutical grade of tizanidine (batch no.: TNZ/QA/0038) and rofecoxib (batch no.: RXB/FP/01) were kindly supplied as a gift sample by Sun Pharma Ltd., Gujarat, India, used without further purification and certified to contain 99.75% (w/w) and 99.52% (w/w), respectively on dried basis. All chemicals and reagents used were of HPLC grade and were purchased from Merck Chemicals, India.

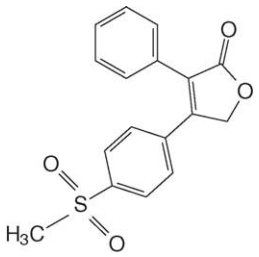

Fig. 2. Structure of rofecoxib.

2.2. Instrumentation and chromatographic conditions

2.2.1. For TLC densitometry

The samples were spotted in the form of bands of width 6 mm with a Camag 100 μl sample (Hamilton, Bonaduz, Switzerland) syringe on precoated silica gel aluminium Plate 60 F-254 (20 cm × 10 cm) with 250 μm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, (Mumbai) using a Camag Linomat IV (Switzerland). The plates were prewashed by methanol and activated at 110 °C for 5 min prior to chromatography. A constant application rate of 0.1 μl/s was employed and space between two bands was 5 mm. The slit dimension was kept at 5 mm × 0.45 mm and 10 mm/s scanning speed was employed. The monochromator bandwidth was set at 20 nm with K 320 cut off filter, each track was scanned thrice and baseline correction was used. The mobile phase consisted of toluene–methanol–acetone (7.5:2.5:1.0, v/v/v) and 15 ml of mobile phase was used per chromatography. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland). Dimensions: length × width × height = 12 cm × 4.7 cm × 12.5 cm. It was saturated (lined on the two bigger sides with filter paper that had been soaked thoroughly with the mobile phase) and the chromatoplate development was carried out in dark with the mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 °C ± 2) at relative humidity of 60% ± 5. The length of chromatogram run was 9 cm and approximately 30 min. Subsequent to the development, TLC plates were dried in a current of air with the help of an air dryer in wooden chamber with adequate ventilation. The flow of air in the laboratory was maintained unidirectional (laminar flow, towards exhaust). Densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorbance mode at 311 nm for all measurements and operated by CATS software (V 3.15, Camag). The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was via peak areas with linear regression.

2.2.2. For HPLC method

The HPLC system consisted of a pump (model Jasco PU 1580, intelligent HPLC pump) with auto injecting facility (AS-1555 sampler) programmed at 20 μl capacity per injection was used. The detector consisted of a UV–vis (Jasco UV 1575) model operated at a wavelength of 235 nm. The software used was Jasco borwin version 1.5, LC-Net II/ADC system. The columns used were Kromasil C-18 (250 mm × 4.6 mm, 5.0 μm) Flexit Jour Laborarories Pvt. Ltd. Pune, India and Finepak SIL-5, C-18