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Validated liquid chromatography method for assay of tizanidine in drug substance and formulated products

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Abstract

A new isocratic stability indicating HPLC method for determination of tizanidine in drug substance and formulated products is described. Chromatographic separation of tizanidine from the related substances and degraded products was achieved with a Hypersil CN column (150 mm × 5.0 mm, 5 μm) using a mobile phase comprising a mixture of an ion-pairing solution of heptanesulphonic acid sodium salt (HAS), methanol and acetonitrile (50:57:18 (v/v)) within 10 min. The flow-rate was 1.0 ml/min and detection was made at 227 nm. The method has good selectivity towards tizanidine, related substances and degraded products. Limits of quantitation for tizanidine and its synthetic intermediates were determined, ranging from 0.051 to 0.54 μg/ml. The linearity range was found to be 2–20 μg/ml ($r = 0.9998$, $n = 5$). Mean recovery for tizanidine from the tablets was from 99.5 to 99.8%. Precision of the method was 1.0% ($n = 9$). The method can be used for routine analysis and the quality control of tizanidine drug substance and its formulated products.

Keywords: Tizanidine; Liquid chromatography; Drug substance; Formulated products

1. Introduction

Tizanidine [5-chloro-4-(2-imidazolin-2-ylamino)-2,1,3-benzothiadiazole], a central alpha-2 adrenoceptor agonist, is a myotonolytic agent used in the treatment of spasticity in patients with cerebral or spinal injury [1–4]. It is an antispastic agent with similar efficacy to that of baclofen and a more favourable tolerability profile. Therefore, tizanidine appears to be an attractive therapeutic alternative for patients with spasticity associated with cerebral or spinal damage. Clinical trials with tizanidine when administered alone have shown that it is safe and effective for spasticity control.

A new tablet formulation for tizanidine was newly developed in our laboratory and an assay method was needed for determination of the drug in the formulation. Yet, there were no methods available for assaying tizanidine and related substances in drug substance and formulated products but a few methods for its pharmacokinetic study [5–7]. Therefore, a specific, rapid and accurate analytical method was required to meet the current analytical needs. The objective of this work is to develop an LC method for determination of tizanidine and related substances in drug substance and formulated products.

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2. Experimental

2.1. Chemicals and reagents

Tizanidine reference standard and tablets containing 2 mg tizanidine in each tablet were provided by Shenyang Pharmtech Institute of Pharmaceuticals (Shenyang, China). HPLC-grade methanol, acetonitrile and glacial acetic acid were obtained from Fisher Scientific (Springfield, NJ, USA). Distilled water was prepared by Milli-Q system (Millipore, MA, USA). HPLC-grade heptanesulphonic acid sodium salt (HAS) was used. All other chemicals used were of analytical grade unless otherwise indicated.

2.2. Apparatus and chromatographic conditions

HP 1100 series chromatographic apparatus (Agilent, CA, USA) was used for this work. Data were collected and processed with EChrom Workstation. Chromatographic separation was performed on a Hypersil CN column (150 mm × 5.0 mm, 5 μm). The mobile phase comprising a mixture of an ion-pairing solution, methanol and acetonitrile (50:57:18 (v/v)) was delivered at a flow-rate of 1.0 ml/min. The ion-pairing solution was made by dissolving 2.5 ml of the aqueous solution of 0.05 mol/l heptanesulphonic acid sodium salt (HAS) and 0.8 ml of triethylamine in 800 ml of water, adjusting the pH to 3.3 with glacial acetic acid prior to dilution to 1000 ml with water. The mobile phase was filtered through a 0.45 μm membrane filter and degassed. Chromatography was performed at ambient temperature and detection was made at 227 nm. The injected volume was 20 μl.

2.3. Preparation of stock and standard solutions

A stock solution of tizanidine was prepared in mobile phase at 400 μg/ml. Five millilitre of stock solution was quantitatively transferred into a 50 ml volumetric flask and made to volume with mobile phase. Standard solutions were prepared by dilution of the diluted stock solution with mobile phase to give solutions containing tizanidine in the concentration range of 2–20 μg/ml.

2.4. Preparation of the solution of a synthetic mixture

The solution of a synthetic mixture of tizanidine and its five precursors/intermediates was made as follows: firstly, dissolve appropriate amount of tizanidine and each of the five precursors/intermediates individually with the mobile phase to achieve the concentration of 100 μg/ml, and then accurately transfer of 1 ml of each of the previously made solutions into a 10 ml volumetric flask and mix well.

2.5. Sample preparation

Twenty tablets were accurately weighed and finely powdered. An accurately weighed portion of the powder equivalent to 2 mg tizanidine was transferred to a 50 ml volumetric flask. After, about 20 ml of the mobile phase were added to the flask, sonicated for 5 min, brought to volume with mobile phase, and filtered. The first 10 ml of the filtrate were rejected, and 2.0 ml of the following filtrate were quantitatively transferred into a 10 ml volumetric flask, and dilute to volume with mobile phase.

3. Results and discussion

3.1. Method development

During our method development, top priority was given to complete separation of tizanidine from its related substances such as its synthetic precursors/intermediates and forcibly degraded products under the stress conditions of direct fire, acid, base and oxidation, which completed a library of compounds used to investigate system selectivity. The structures of tizanidine and its synthetic precursors/intermediates are as shown in Fig. 1.

Since tizanidine exhibits lipophilic and basic properties, it becomes ionised in acidic conditions and soluble in water. Therefore, the mobile phase containing ion-pairing reagent and at a low pH value will favour the retention and separation of the drug. Two ion-pairing reagents were tried during our preliminary work, heptanesulphonic acid sodium salt (HAS) and octanesulphonic acid sodium salt (OAS). HAS was finally used as the ion-pairing reagent in our study

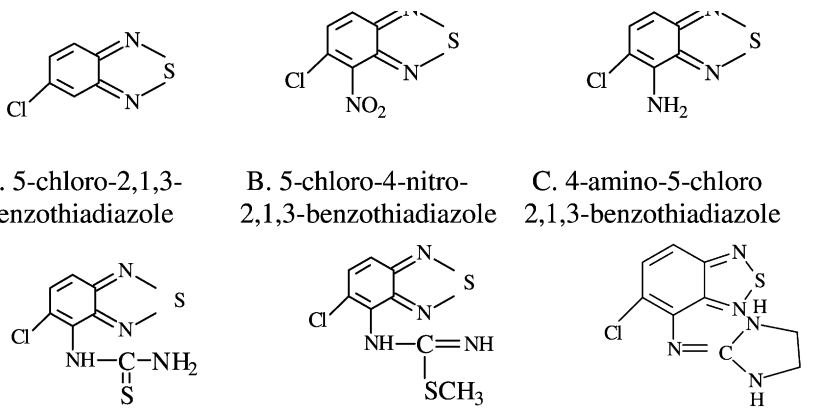

Fig. 1. Structures of tizanidine and its related compounds.

because it offered the good separation and reasonable retention of tizanidine and its related substances. Small amount of triethylamine was added to avoid peak tailing of the drugs.

Acetonitrile and methanol are commonly used organic solvents in HPLC. They have different effects on the solvation of the analytes and on the symmetry and selectivity of the peaks. At first, the mixture of ion-pairing solution and methanol were used as the mobile phase, but did not achieve the desirable separation, i.e. reasonable retention, high selectivity and good symmetry of the analytes. Afterwards, acetonitrile was added into the mobile phase to improve the peak shapes of the analytes and also to reduce the column pressure. Finally, a mobile phase consisting of a mixture of an ion-pairing solution, methanol and acetonitrile (50:57:18 (v/v)) was adopted, which could not only achieve the desirable retention and best resolution between the tizanidine and its related substances but also maintain symmetrical peaks.

Finally, the influence of the flow-rate of the mobile phase was studied. The retention times and chromatographic resolution decreased when the flow-rate increased. A flow-rate of 1.0 ml/min was selected because it produced reasonable retention times and resolution for all analytes. Under the given conditions, the retention time for tizanidine was about 7.0 min. Total time of analysis was <10 min. The maximum absorption of tizanidine in the mobile phase was found to be 227 and 318 nm. Since the absorbance at 227 nm was much higher than that at 318 nm, the wavelength 227 nm was chosen as the detection wavelength.

3.2. Method validation

3.2.1. Sample stability

Stability of tizanidine in the mobile phase was determined by injecting the sample solutions of tizanidine drug substance and tablets at 0, 4, 8, 12 and 24 h of post-preparation at room temperature with protection of samples from direct sunlight. No peaks corresponding to the degradation products were observed and there was no significant change in the drug's peak area. Tizanidine was found to be stable in the mobile phase for at least 24 h.

3.2.2. Selectivity and specificity

Selectivity of the described method was determined by analysing both the synthetic mixture of tizanidine with its five precursors/intermediates (Fig. 1) and forcefully degraded tablet samples. The chromatogram of the synthetic mixture is shown in Fig. 2, which shows good resolution between most of the analytes except that between peaks 4 and 5.

Forced degradation studies were performed to provide an indication of the stability indicating property

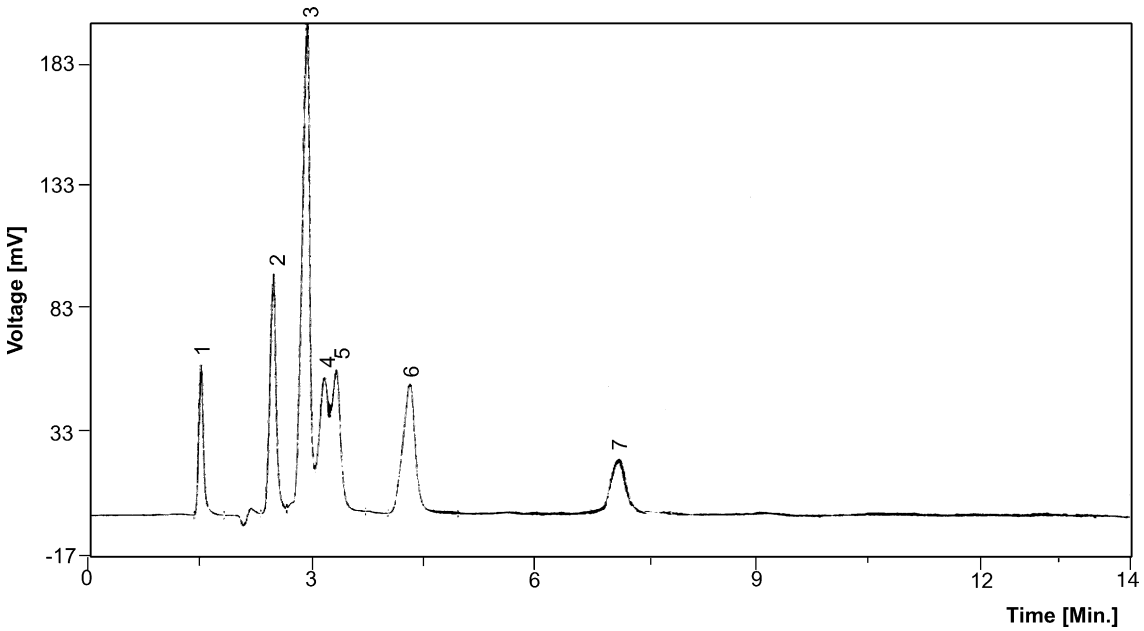

Fig. 2. HPLC chromatogram of the synthetic mixture of tizanidine with its precursors/intermediates. Peaks: (1) an isomer of *S*-methyl-*N*-(5-chloro-2,1,3-benzo-thiadiazol-4-yl)isothiurone; (2) *N*-(5-chloro-2,1,3-benzo-thiadiazol-4-yl)thiourea; (3) 4-amino-5-chloro-2,1,3-benzo-thiadiazole; (4) 5-chloro-2,1,3-benzothiadiazole; (5) 5-chloro-4-nitro-2,1,3-benzothiadiazole; (6) *S*-methyl-*N*-(5-chloro-2,1,3-benzo-thiadiazol-4-yl)isothiurone; (7) tizanidine.

and specificity of the proposed method. Intentional degradation was achieved by exposing the drug substance and tablets to stress conditions of direct fire, acid (0.1 mol/l of HCl), base (0.1 mol/l of NaOH) and oxidation (3% H_2O_2) in order to test the ability of the proposed method to separate tizanidine from the degradation products. In each case, 0.4 mg/ml of tizanidine stock solution was combined with acid, base or peroxide solution and allowed to stand at room temperature or heated at 90 °C for 30 min or more according to the extent of degradation. Samples were degraded to levels where the content of tizanidine in the sample was lowered to <90.0% of the level present in the unstressed time zero reference solution. Chromatograms of degraded samples of tizanidine tablets by direct fire, acid, base and oxidation were individually shown in Fig. 3(A–D), indicating that most of the degradation products could be eluted within 6 min and well resolved from tizanidine. The retention time for tizanidine was about 7.0 min. The proposed method was found to be specific to tizanidine and its related substances.

To demonstrate the specificity of the method, placebo formulations, containing the tablet ingredients other than the drug, were also determined to see if any interference from the excipients existed. A clean chromatogram was obtained which shows there was no interference from the excipients. The ability of the method to separate the drug from its degradation products and the non-interference from the tablets indicates the specificity of the method.

Table 1
LOQ for tizanidine and its five synthetic precursors/intermediates

Substances	LOQ (μg/ml)
5-Chloro-2,1,3-benzothiadiazole	0.32
5-Chloro-4-nitro-2,1,3-benzothiadiazole	0.54
4-Amino-5-chloro-2,1,3-benzothiadiazole	0.18
N-(5-Chloro-2,1,3-benzo-thiadiazole-4-yl)thiourea	0.20
S-Methyl-*N*-(5-chloro-2,1,3-benzothiadiazol-4-yl) isothiurone	0.28
Tizanidine	0.051

3.2.3. Limit of quantitation

Limits of quantitation (LOQ) of the HPLC method for tizanidine (F) and its five synthetic precursors/intermediates separately marked (A–E) as shown in Fig. 1 was established at a signal/noise ratio of 10, which were experimentally verified by six injections of each of the substances at LOQ concentrations. The results are shown in Table 1 indicating the sensitivity of the method.

3.2.4. Linearity

Linearity was evaluated by determining five standard working solutions containing 2–20 μg/ml of tizanidine in triplicate. Peak area (*A*) and concentration

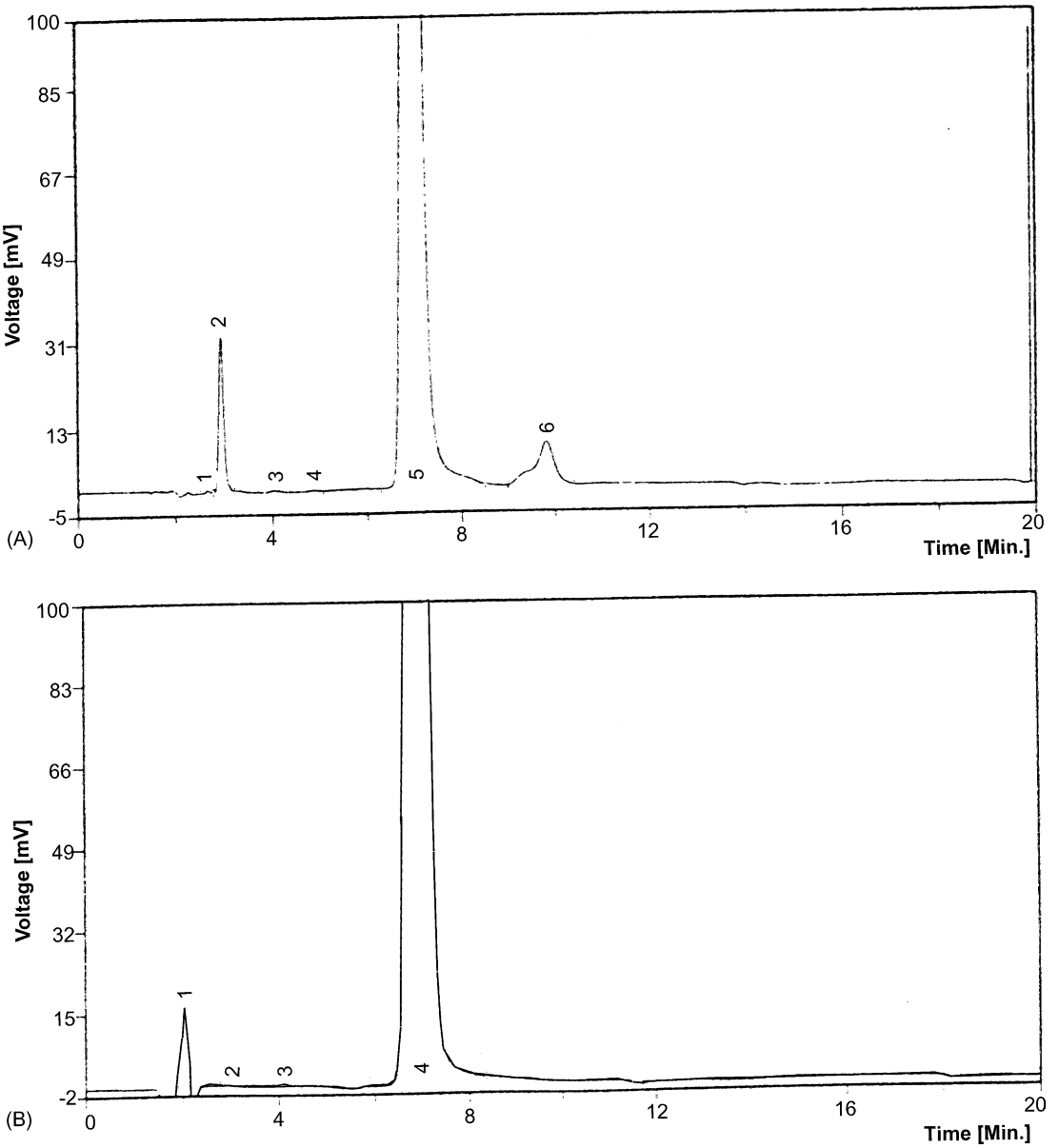

Fig. 3. HPLC chromatograms of tizanidine and its degraded products by: (A) thermal degradation; (B) acid degradation; (C) base degradation; and (D) oxidative degradation.

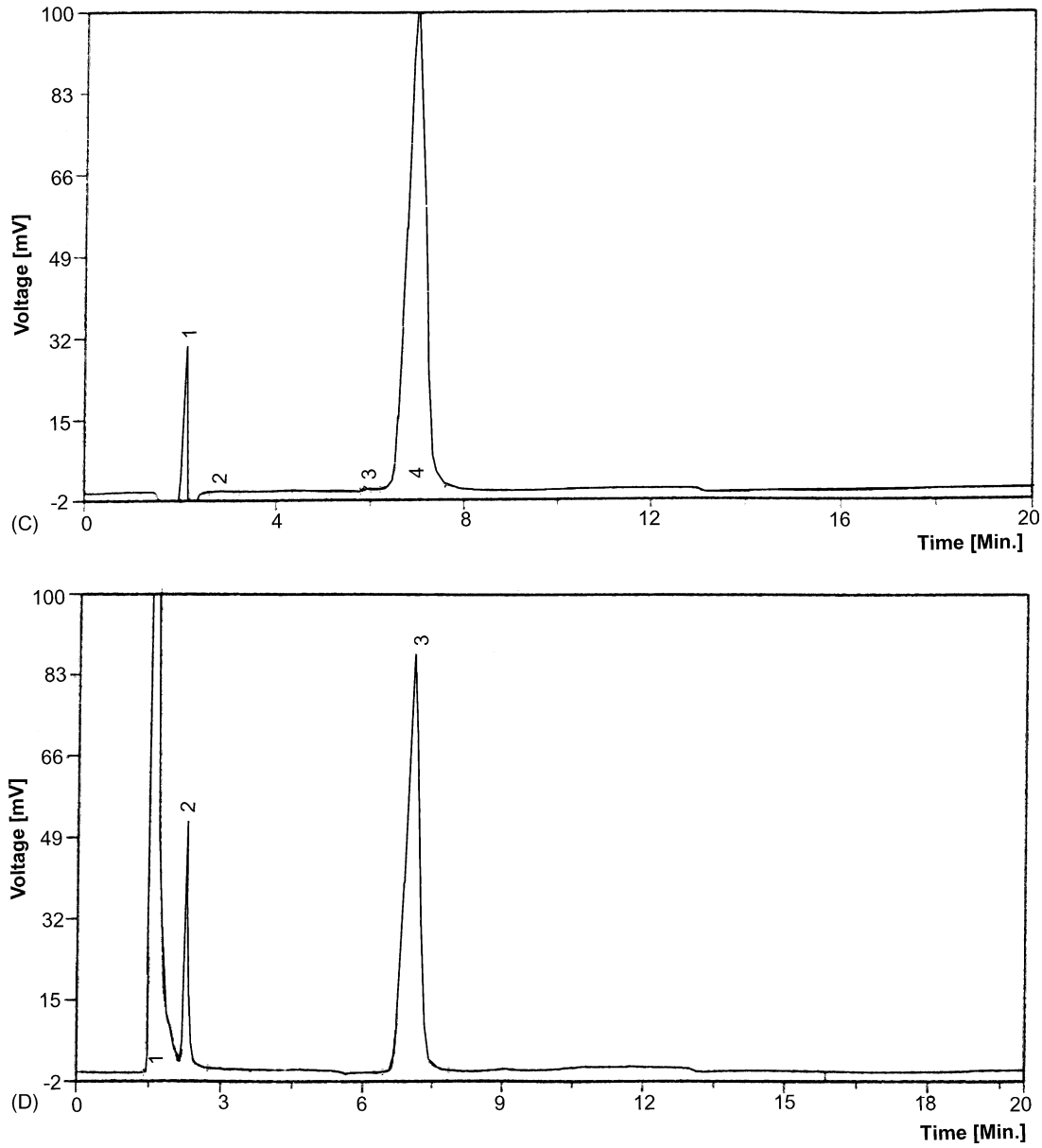

Fig. 3. (*Continued*).

(*C*) was subjected to least-squares linear regression analysis to calculate the calibration equation and correlation coefficients. The regression equation obtained was $A = -569.7 + 1622.4C$ ($r = 0.9998$, $n = 5$). The linear range was from 2 to 20 μg/ml. The results show that there was an excellent correlation between peak area and concentration of tizanidine in the concentration range tested.

3.2.5. Accuracy

Accuracy of the described method was determined with the tablets, applying the method to synthetic

mixtures of excipients to which known amount of tizanidine corresponding to 80, 100 and 120% of label claim had been added. At each level of the amount, five determinations were performed. Mean recovery for tizanidine from the tablets was (mean ± S.D.) 99.5 ± 1.2%, 99.7 ± 1.0% and 99.8 ± 0.9% for each level of the amount, which indicated good accuracy of the method for determination of tizanidine in the tablets.

3.2.6. Precision

Precision of the method was determined with the product. An amount of the product powder equivalent to 100% of label claim of tizanidine was accurately weighed and assayed in nine replicate determinations. Acceptable precision was achieved with the method as revealed by relative standard deviation data (R.S.D. = 1.0%, $n = 9$).

3.3. Method application

The validated HPLC method was applied to determination of tizanidine in drug substance and tablets. Two batches of drug substance of tizanidine were determined for assay and related substances. For both the batches, the amount of the drug was >99.0%, and the total amount of the related substances was <1%. Three batches of tizanidine tablets were assayed. The assay results, expressed as the percentage of the label claim, were 98.9, 97.5 and 97.6%, respectively, indicating that the amount of tizanidine in the tablets met the requirements (90–110% of the label claim).

4. Conclusion

The LC method developed for tizanidine is highly selective, accurate and precise. The validated method may be regarded as a stability indicating one, which proved to be suitable both for assessing the stability of tizanidine in the drug substance and formulated products and for routine analysis of tizanidine in ordinary laboratories.

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