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Stability-indicating HPTLC determination of tizanidine hydrochloride in bulk drug and pharmaceutical formulations

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Abstract

A simple, selective, precise and stability-indicating high-performance thin-layer chromatographic method of analysis of tizanidine hydrochloride both as a bulk drug and in formulations was developed and validated. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of toluene–acetone–ammonia (5:5:0.1, v/v/v). This system was found to give compact spots for tizanidine hydrochloride (R_f value of 0.32 ± 0.01). Tizanidine hydrochloride was subjected to acid and alkali hydrolysis, oxidation and photodegradation. Also, the degraded product was well separated from the pure drug. Densitometric analysis of tizanidine hydrochloride was carried out in the absorbance mode at 315 nm. The linear regression analysis data for the calibration plots showed good linear relationship with $r^2 = 0.9922$ in the concentration range 300–1000 ng per spot. The mean value of correlation coefficient, slope and intercept were 0.9922 ± 0.002, 0.064 ± 0.001 and 38.09 ± 1.71, respectively. The method was validated for precision, recovery and robustness. The limits of detection and quantitation were 88 and 265 ng per spot, respectively. The drug does not undergo degradation under acidic and basic conditions. The samples degraded with hydrogen peroxide showed additional peak at R_f value of 0.12. This indicates that the drug is susceptible to oxidation. Statistical analysis proves that the method is repeatable and selective for the estimation of said drug. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

Keywords: Tizanidine hydrochloride; HPTLC; Stability indicating; Degradation

1. Introduction

Tizanidine hydrochloride 5-chloro-4-(2-imidazolin-2-yl-amino)-2,1,3-benzothiadiazole hydrochloride (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

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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 hydrochloride 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 the estimation of tizanidine in bulk and its dosage form. An RP-HPLC method for the 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.

The International Conference on Harmonization (ICH) guideline entitled 'stability testing of new drug substances and products' requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance [9]. Susceptibility to oxidation is one of the required tests. Also, the hydrolytic and the photolytic stability are required. An ideal stability-indicating method is one that quantifies the drug per se and also resolves its degradation products. Nowadays, HPTLC is becoming a routine analytical technique due to its advantages [10–12]. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis.

The aim of this work is to develop an accurate, specific, repeatable and stability-indicating method for the determination of tizanidine hydrochloride in the presence of its degradation products and related impurities as per ICH guidelines [13].

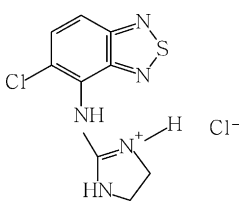

Fig. 1. Structure of tizanidine hydrochloride.

2. Experimental

2.1. Materials

Tizanidine hydrochloride was a gift from Sun Pharma India Ltd. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

2.2. HPTLC instrumentation

The samples were spotted in the form of bands of width 6 mm with a Camag microlitre syringe on precoated silica gel aluminium plate 60F-254 (20 cm × 10 cm with 250 μm thickness, E. Merck, Germany) using a Camag Linomat IV (Switzerland). 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 mobile phase consisted of toluene–acetone–ammonia (5:5:0.1, v/v/v). Linear ascending development was carried out in twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 15 min at room temperature. The length of chromatogram run was 8 cm. Subsequent to the development, TLC plates were dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 315 nm. The source of radiation utilized was deuterium lamp.

2.3. Calibration curves of tizanidine hydrochloride

A stock solution of tizanidine hydrochloride (100 ng/μl) was prepared in methanol. Different volumes of stock solution, 3, 4, 5, 6, 7, 8, 9 and 10 μl, were spotted on TLC plate to obtain concentrations of 300, 400, 500, 600, 700, 800, 900, 1000 ng per spot of tizanidine hydrochloride, respectively. The data of peak area vs. drug concentration were treated by linear least-square regression

analysis. Linearity was also determined over the range 1000–6000 ng per spot.

2.4. Method validation

2.4.1. Precision

Repeatability of sample application and measurement of peak area were carried out using seven replicates of the same spot (600 ng per spot of tizanidine hydrochloride). It showed very low %RSD of peak area of tizanidine hydrochloride. The intra- and inter-day variation for the determination of tizanidine hydrochloride was carried out at three different concentration levels of 400, 800 and 2000 ng per spot.

2.4.2. Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition of toluene–acetone–ammonia (4.9:5.1:0.1 and 5.1:4.9:0.1, v/v/v) were tried at three different concentration levels of 400, 800 and 2000 ng per spot.

2.4.3. Limit of detection and limit of quantification

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank methanol was spotted six times following the same method as explained in Section 2.2. The signal-to-noise ratio was determined.

2.4.4. Recovery studies

The analyzed samples were spiked with extra 50, 100 and 150% of the standard tizanidine hydrochloride and the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulations.

2.5. Analysis of the marketed formulation

To determine the content of tizanidine hydrochloride in tablets (label claim: 2 mg per tablet), the tablets were powdered and powder equivalent to 2 mg of tizanidine hydrochloride was weighed. The drug from the powder was extracted with methanol. To ensure complete extraction of the drug, it was sonicated for 30 min and volume was made up to 10 ml. The resulting solution was centrifuged at 3000 rpm for 5 min and supernatant was analyzed for drug content. 3 μl of the filtered solution (600 ng per spot) was applied on TLC plate followed by development and scanning as described in Section 2.2. The analysis was repeated in triplicate. The possibility of excipient interference in the analysis was studied.

2.6. Forced degradation of tizanidine hydrochloride

A stock solution containing 50 mg tizanidine hydrochloride in 50 ml methanol was prepared. This solution was used for forced degradation.

2.6.1. Preparation of acid- and base-induced degradation product

To 20 ml of methanolic stock solution, 5 ml of 5 N HCl and 5 ml of 5 N NaOH were added separately. These mixtures were refluxed for 6 h at 90 °C. The forced degradation in acidic and basic media was performed in the dark in order to exclude the possible degradative effect of light. The resultant solution (800 ng per spot) was applied on TLC plate and the chromatograms were run as described in Section 2.2.

2.6.2. Preparation of hydrogen peroxide-induced degradation product

To 20 ml of methanolic stock solution, 5 ml of hydrogen peroxide (30.0%, v/v) was added. The solution was heated in boiling water bath for 6 h to remove completely the excess of hydrogen peroxide. The resultant solution (800 ng per spot) was applied on TLC plate and the chromatograms were run as described in Section 2.2.

2.6.3. Photochemical degradation product

The photochemical stability of the drug was also studied by exposing the stock solution to direct sunlight for 24 h. The resultant solutions were appropriately diluted and 800 ng per spot was applied on TLC plate and chromatograms were run as described in Section 2.2.

Table 1
Linear regression data for the calibration curves[a]

Linearity range (ng)	$r \pm$S.D.	Slope $\pm$S.D.	Confidence limit of slope[b]	Intercept $\pm$S.D.	Confidence limit of intercept[b]
300–1000	0.9922 $\pm$ 0.002	0.064 $\pm$ 0.001	$6.29–6.51 \times 10^{-2}$	38.09 $\pm$ 1.71	36.15–40.03
1000–6000	0.9886 $\pm$ 0.002	0.011 $\pm$ 0.0004	$10.5–11.45 \times 10^{-3}$	37.03 $\pm$ 3.40	33.18–40.88

[a] $n = 3$.
[b] 95% confidence limit.

In all degradation studies, the average peak area of tizanidine hydrochloride after application (800 ng per spot) of seven replicates was obtained.

2.7. Detection of the related impurities

The related impurities were determined by spotting higher concentrations of the drug so as to detect and quantify them. Tizanidine hydrochloride (50 mg) was dissolved in 10 ml of methanol, and this solution was termed as sample solution (5 mg/ml). 1 ml of the sample solution was diluted to 100 ml with methanol and this solution was termed as standard solution (0.05 mg/ml). 10 μl of both the standard (500 ng per spot) and the sample solution (50,000 ng per spot) was applied on TLC plate and the chromatograms were run as described in Section 2.2.

3. Results and discussion

3.1. Development of the optimum mobile phase

TLC procedure was optimized with a view to develop a stability-indicating assay method. Both the pure drug and the degraded products were spotted on TLC plates and run in different solvent systems. Initially, toluene–acetone in varying ratios were tried. The mobile phase toluene–acetone (5:5, v/v) gave good resolution with R_f value of 0.32 for tizanidine hydrochloride but typical peak nature was missing. Also, the spot for tizanidine hydrochloride was slightly diffused. Addition of 0.1 ml ammonia to the above mobile phase improved the spot characteristics. Finally, the mobile phase consisting of toluene–acetone–ammonia (5:5:0.1, v/v/v) gave a sharp and symmetrical peak. Well-defined spots were obtained when the chamber was saturated with the mobile phase for 15 min at room temperature.

3.2. Calibration curves

The linear regression data for the calibration curves ($n = 3$) as shown in Table 1 showed a good linear relationship over the concentration range 300–1000 ng per spot as well as over concentration range 1000–6000 ng per spot. No significant difference was observed in the slopes of standard curves (ANOVA, $P > 0.05$).

Table 2
Intra- and inter-day precision of HPTLC method[a]

Amount (ng per spot)	Intra-day precision			Inter-day precision		
	S.D. of areas	%RSD	SE	S.D. of areas	%RSD	SE
400	0.60	0.84	0.30	0.71	0.90	0.46
800	2.10	2.70	0.94	2.21	2.95	1.20
2000	1.70	2.40	0.76	1.84	2.49	1.04

[a] $n = 6$.

Table 3
Recovery studies[a]

Excess drug added to the analyte (%)	Theoretical content (ng)	Recovery (%)	%RSD	SE
0	400	99.70	0.84	0.24
50	600	101.36	2.20	0.66
100	800	98.76	1.20	0.36
150	1000	99.21	1.90	0.58

[a] $n = 6$.

3.3. Validation of the method

3.3.1. Precision

The repeatability of sample application and measurement of peak area were expressed in terms of %RSD and found to be 1.12 and 0.05, respectively. The results depicted in Table 2 revealed intra- and inter-day variation of tizanidine hydrochloride at three different concentration levels of 400, 800 and 2000 ng per spot.

3.3.2. Robustness of the method

The low values of %RSD obtained after introducing small changes in mobile phase composition indicated robustness of the method.

3.3.3. LOD and LOQ

The signal-to-noise ratios of 3 and 10 were considered as LOD and LOQ, respectively. The LOD and LOQ were found to be 88 and 265 ng per spot, respectively.

3.3.4. Recovery studies

The proposed method when used for extraction and subsequent estimation of tizanidine hydrochloride from pharmaceutical dosage forms after spiking with 50, 100 and 150% of additional drug afforded recovery of 98–102% as listed in Table 3.

3.4. Analysis of the marketed formulation

A single spot at R_f 0.33 was observed in the chromatogram of the drug samples extracted from tablets. There was no interference from the excipients commonly present in the tablets. The drug content was found to be 99.4% with a %RSD of 0.52%. It may therefore be inferred that degradation of tizanidine hydrochloride had not occurred in the marketed formulations that were analyzed by this method as shown in Table 4. The low %RSD value indicated the suitability of this method for routine analysis of tizanidine hydrochloride in pharmaceutical dosage forms.

3.5. Stability-indicating property

The chromatogram of the acid- and base-degraded samples for tizanidine hydrochloride showed only the spots of the pure drug. No significant change was observed in drug concentration in both cases. The samples degraded with hydrogen peroxide (Fig. 2) showed additional peak at R_f value of 0.12. The spot of degraded product was well resolved from the drug spot. The photodegraded sample showed no additional peak. The results are listed in Table 5.

Table 4
Applicability of HPTLC method for the analysis of the pharmaceutical formulations ($n = 6$)

Drug	Label claim	Drug content (%)	%RSD	SE	t	F	t^a	F^a
Tizanidine hydrochloride	2 mg	101.12 ± 0.55	0.54	0.22	0.054	1.083	2.57	5.05

[a] Theoretical values for t and F.

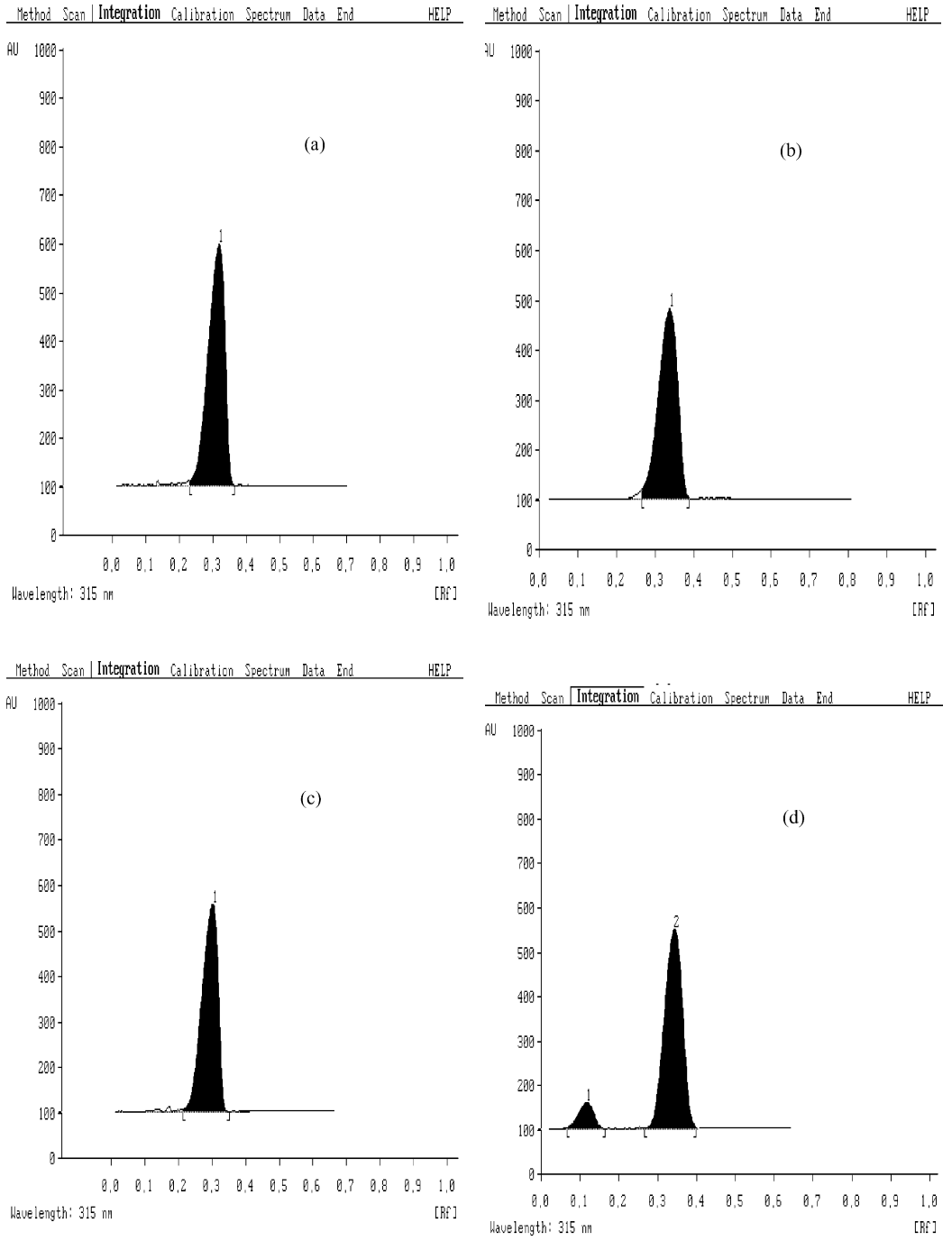

Fig. 2. Chromatograms of tizanidine and its degraded products: (a) pure drug: peak 1 (R_f: 0.3) is of tizanidine; (b) acid-treated: peak 1 (R_f: 0.3) is of tizanidine; (c) base-treated: peak 1 (R_f: 0.3) is of tizanidine; (d) degraded with hydrogen peroxide: peak 1 (R_f: 0.12) is of degraded product, peak 2 (R_f: 0.3) is of tizanidine; (e) photo-treated: peak 1 (R_f: 0.3) is of tizanidine.

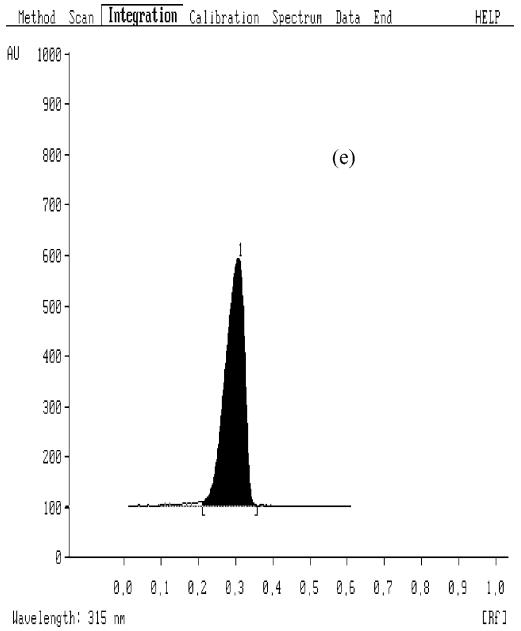

Fig. 2 (*Continued*)

Table 5
Degradation of tizanidine hydrochloride

Condition	Time (h)	Recovery (%)	R_f value of degradation products
Acid 5 N HCl (refluxed)	6	100	None detected
Base 5 N NaOH (refluxed)	6	100	None detected
H_2O_2 30 vol. (refluxed)	6	88.6	0.12
Day light	24	100	None detected

3.6. Detection of the related impurities

The spots other than the principal spot and the spot of the starting point from the sample solution were not intense than the spot from the standard solution. The sample solution showed one additional spot at R_f 0.54 (Fig. 3). However, the area of the additional spot was found to be much less as compared to the standard solution as indicated in Table 6.

Table 6
Related impurities

Amount of drug (ng per spot)	R_f value	Area
500	0.32	3695.81
Related impurity		
50,000	0.54	2360.2

4. Conclusion

The developed HPTLC technique is precise, specific, accurate and stability indicating. Statistical analysis proves that the method is repeatable and selective for the analysis of tizanidine hydrochloride as bulk drug and in pharmaceutical formulations. The method can be used to determine the purity of the drug available from various sources by detecting the related impurities. It may

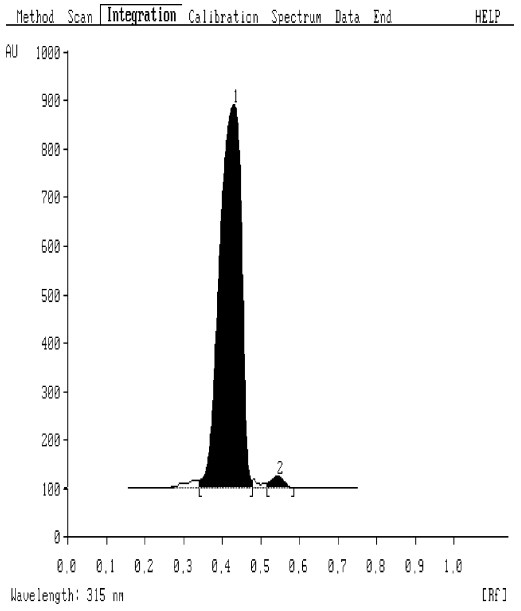

Fig. 3. Chromatogram of tizanidine hydrochloride and its impurity: peak 1 (R_f: 0.4) is of tizanidine hydrochloride; peak 2 (R_f: 0.54) is of impurity.

be extended to study the degradation kinetics of tizanidine hydrochloride and also for its estimation in plasma and other biological fluids. As the method separates the drug from its degradation products, it can be employed as a stability-indicating one.

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