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Simultaneous Estimation of Related Impurities of Tizanidine Hydrochloride in its Active Pharmaceutical Ingradient by Reversed-Phase Liquid Chromatography

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A simple method was developed for qualitative and quantitative estimation of tizanidine hydrochloride and its five structurally related impurities using a reversed-phase liquid chromatography. This method was validated as per ICH guidelines for 'validation for analytical procedures' Q2B. The liquid chromatography was set up in UV detection mode at 310 nm. A separation was achieved between tizanidine and all five impurities in a gradient programming using YMC Pack Pro C_{18} (250 mm × 4.6 mm), 5 µ column at 1 mL/min flow rate. This method was established to be linear in a concentration range of (0.5-4.0 µg/mL) for all the components considered in this study. Limit of detection (LOD) and limit of quantification (LOQ) values were found to be (0.1-0.15 µg/mL) and (0.3-0.45 µg/mL) for tizanidine hydrochloride and its five related impurities, respectively. Recoveries of all spiked impurities in tizanidine hydrochloride were in a range of (95- 105 %). Robustness and sample solution stability studies demonstrated the ruggedness of this method. This method is simple, sensitive and provides precisely accurate results.

Key Words: Tizanidine hydrochloride, Active pharmaceutical ingradient, Reversed-phase high performance liquid chromatography.

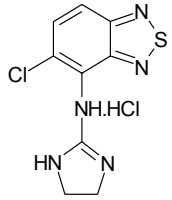
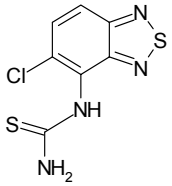
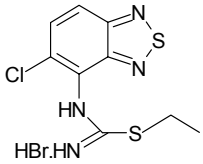
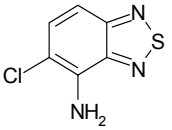
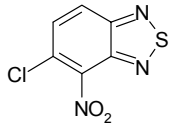
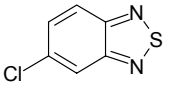
INTRODUCTION

Tizanidine hydrochloride, chemically is 5-chloro-4-(2-imidazoline-2-ylamino)-2,1,3-benzothiadiazole mono-hydrochloride. It is a muscle relaxant drug, which has been derived from imidazole family of derivatives and extensively used in the treatment of patients with multiple sclerosis or spinal cord disorders[1].

Literature survey reveals that HPLC methods for simultaneous determination of tizanidine and other NSAIDS have been reported[2-4] but not for determination of tizanidine and its five related impurities described in this study. Tizanidine hydrochloride is listed in United States Pharmacopoeia[5],

which describes a related substances HPLC method for the quantitative estimation of three related impurities of tizanidine hydrochloride, which are different than the other five impurities studied in this research work. Moreover, this method is not useful for the separation of the five related impurities. Therefore, the aim of this study was to develop a simple, precise and accurate reversed-phase HPLC method for simultaneous separation and estimation of tizanidine and its five related impurities. The five impurities described in this research paper are structurally similar to tizanidine with imidazole as their structural base (Table-1) and are very likely to be introduced in Tizanidine hydrochloride in the course of synthesis.

TABLE-1
CHEMICAL NAME, STRUCTURE OF TIZANIDINE HYDROCHLORIDE AND ITS FIVE RELATED IMPURITIES

Name	Structure	Name	Structure
Tizanidine hydrochloride 5-chloro-4-(2-imidazoline-2-ylamino)-2,1,3-benzothiadiazole mono-hydrochloride		**Impurity A** N-(5-Chloro-2,1,3-benzothiadiazole-4-yl)thiourea	
Impurity B S-Ethyl-N-(5-chloro-2,1,3-benzothiadiazole-4-yl) isothiouronium bromide		**Impurity C** 4-Amino-5-chloro-2,1,3-benzothiadiazole	
Impurity D 5-Chloro-4-nitro-2,1,3-benzothiadiazole		**Impurity E** 5-Chloro-2,1,3-benzothiadiazole	

EXPERIMENTAL

A liquid chromatography hardware system used was a Lachrom HPLC (Merck-Hitachi, Japan). This consisted of a quaternary low pressure gradient pump, model L-2130, an auto-sampler with thermo conditioning device, model L-2200, a UV-fixed wavelength detector, model L-2400 and a column oven, model L-2300. Integration and data storage was done on EZchrom Elite software, version 3.1.3. YMC-Pack Pro C18, 250 × 4.6 mm, 5 µ (YMC Co. Ltd. USA) column was used as stationary phase.

Reference standards of tizanidine hydrochloride and impurity-A, B, C, D and E of certified purity were procured from Merck (India) Ltd.

Acetonitrile and water (both HPLC grade) and potassium dihydrogen phosphate anhydrous and orthophosphoric acid (both analytical grade), were purchased from Merck (India) Ltd.

Preparation of mobile phase: Preparation of mobile phase-A: 850 mL 20 mM KH_2PO_4 (anhydrous) was mixed with 150 mL of acetonitrile (85:15 v/v): pH = 3.0 was adjusted with orthophosphoric acid. Preparation of mobile phase-B: 200 mL 20 mM KH_2PO_4 (anhydrous) was mixed with 800 mL of acetonitrile (20:80 v/v): pH = 3.0 was adjusted with orthophosphoric acid. Both the mobile phases were filtered through 0.45 µ membrane filter (millipore) and degassed by sonication before use.

Standard stock solution: 20 mg of tizanidine hydrochloride, impurity-A, B, C, D and E were weighed separately in six different 100 mL volumetric flasks. They were dissolved with 20 mL of the mobile phase-A (diluent) and diluted up to the mark with the same. The resulting solutions in 100 mL volumetric flasks contain 200 µg/mL solution of tizanidine hydrochloride and impurities A, B, C, D and E, respectively.

Working standard solution: A working standard solution was prepared from standard stock solution. 1 mL each of the above standard stock solutions was transferred into a 100 mL volumetric flask and diluted up to the mark with the diluent. Resulting solution contained 2 µg/mL of tizanidine hydrochloride and impurities A, B, C, D and E. All sample preparations were performed at 25 °C temperature and were protected from direct light.

Chromatographic conditions: The HPLC system was programmed in a linear gradient mode. A linear gradient was programmed from 100 % to 0 % mobile phase-A and 0 % to 100 % mobile phase-B in 40 min. Initial condition of 100 % mobile phase-A was achieved at 41st min and run time was continued till 50 min. Mobile phase was maintained at a flow rate of 1.0 mL/min. Injection volume was 20 µL and 310 nm was used as UV detector wavelength. Mobile phase-A was used as a diluent for all sample preparations.

Method validation: The proposed analytical method was subjected to various validation parameters to judge its merit for its intended use as per ICH guidelines for 'validation for analytical procedures' Q2B[6]. The proposed method was validated for limit of detection (LOD), limit of quantification (LOQ), linearity, range, specificity, precision, accuracy, robustness and sample solution stability.

System suitability test: A system suitability test[5] was conducted on six replicate injections of the working standard solution containing tizanidine and impurities-A to E. The various parameters such as theoretical plates per meter, tailing factor, resolution between closely eluting peaks of tizanidine and all impurities were obtained. The results are tabulated under Table-2.

TABLE-2
SYSTEM SUITABILITY PARAMETERS

Parameters	Tizanidine HCl	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E
Retention time (min)	5.03	11.26	12.87	25.72	28.72	30.12
Asymmetry	1.44	1.15	1.13	1.06	1.07	1.04
Resolution	–	31.77	8.15	57.07	12.24	5.47
Theoretical plates	10148	54675	65345	170688	228126	197200
%RSD of the peak areas	0.83	0.68	0.84	1.34	1.04	0.91

Limit of detection (LOD) and limit of quantification (LOQ): The LOD values for tizanidine, impurity-A, B, C, D and E were 0.15, 0.15, 0.15, 0.15, 0.1 and 0.1 µg/mL, respectively. The LOQ values were 0.45, 0.45, 0.45, 0.45, 0.3 and 0.3 µg/mL, respectively.

Linearity and range: A linearity study was carried out in the concentration range of 0.5, 1.0, 2.0, 3.0 and 4.0 µg/mL of tizanidine and impurity-A, B, C, D and E. The selection of concentration range covered 25, 50, 100, 150 and 200 % concentration of individual impurity with respect to tizanidine concentration of 2 mg/mL. The responses were measured as peak areas and were plotted against concentration in µg/mL. The coefficient of correlation for individual component was found > 0.99, which indicates that system is linear even at very low concentration level. The results of linearity are depicted in Table-3.

TABLE-3
LINEARITY OF RESPONSE FOR TIZANIDINE HYDROCHLORIDE AND RELATED IMPURITIES

Concentration (µg/mL)	Tizanidine HCl	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E
0.5	99076	88571	88007	79677	142087	153784
1.0	198685	184996	177026	157510	286647	310774
2.0	407971	403023	365001	325508	591866	647897
3.0	617297	596120	548172	491238	892395	944937
4.0	828673	810972	734208	660018	1193727	1256048
Slope	208802	206322	184916	166202	301146	315151
Intercept	-8143	-16541	-5841	-6233	-11063	871
Correlation coefficient(r^2)	1	0.9997	1	0.9999	1	0.9996

Accuracy: Accuracy of the chromatographic method was established by using standard addition technique. A known amount of impurity standards A, B, C, D and E were spiked in tizanidine hydrochloride standard solution in a concentration level of 0.05, 0.10 and 0.15 % with respect to tizanidine hydrochloride. The analysis was repeated three times per concentration level. % Recovery was calculated by external standard quantification

method, which was found to be in a range of 90 to 100 % for individual impurity-A, B, C, D and E.

Specificity: Specificity of the method was assessed by spiking tizanidine standard solution (2 mg/mL) with impurity standard mixture-A, B, C, D and E in a concentration level of 2 µg/mL *i.e.* 0.1 % impurity concentrations[6] with respect to tizanidine concentration. Fig. 1 demonstrates ability of the method to selectively determine any individual impurity in the presence of tizanidine and any other impurities that are likely to be present.

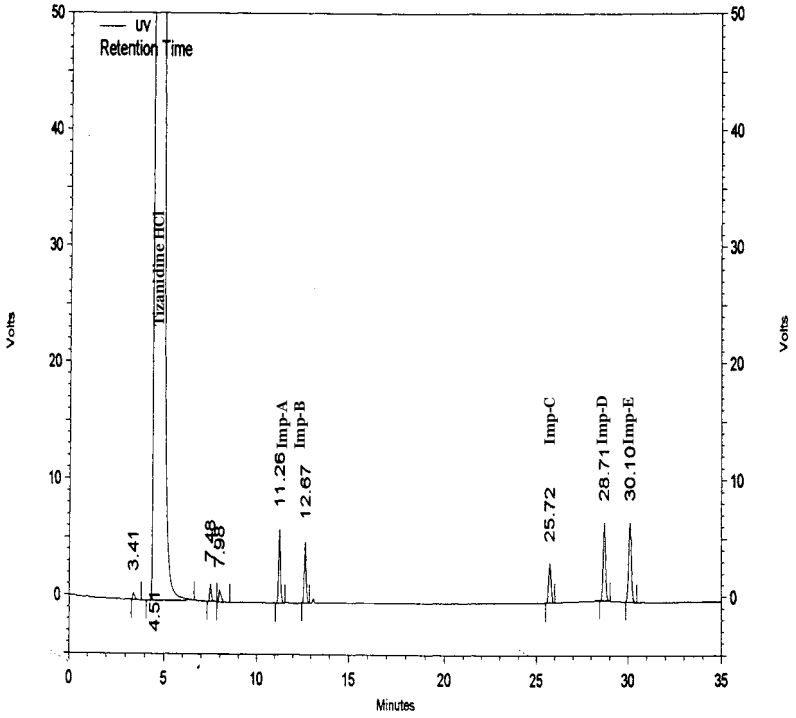


Fig. 1. Specificity

Precision: Precision of the proposed method was determined for the following parameters.

Instrumental precision: Instrumental precision was established by six replicate injections of working standard solution (containing 2 µg/mL of tizanidine hydrochloride, impurity-A, B, C, D and E) in the liquid chromatography system. The % RSD value of individual peak area obtained was less than 5.0 %.

Method precision (analysis repeatability): Method repeatability was established by analyzing a tizanidine hydrochloride standard solution (2 mg/mL) spiked with impurities-A, B, C, D and E at concentration level of

0.05 % (1 µg/mL), 0.1 % (2 µg/mL) and 0.15 % (3 µg/mL) in triplicate. The content of individual impurity was calculated by external standard method. The content of individual impurity spiked in the sample was determined. Average % RSD of three-concentration levels was found to be less than 1.0 %.

Intermediate precision (ruggedness): Intermediate precision of the chromatographic method was determined by analyzing tizanidine hydrochloride standard spiked with impurity A, B, C, D and E at concentration level of 0.1 % with respect to tizanidine hydrochloride on different days and on different instruments. % Recovery of individual impurity was compared for the reproducibility of results. % RSD value between any of these two results was less than 2.0 %.

Robustness: Robustness of the chromatographic method was demonstrated, by making some small but deliberate changes in the method parameters such as pH and flow rate of the mobile phase. It was observed that none of the changes showed significant effect on above system suitability parameters, which proves the robustness of the method.

Stability of sample solution: Stability study of sample solution was established by analyzing it after 24 h. The results indicate no significant change in the estimation results of the same solution thus confirming the stability of the drug in the diluents used for the analysis.

RESULTS AND DISCUSSION

Comprehensive validation study demonstrates an ability of the method to resolve and quantify five structurally similar impurities, which are likely to generate in the course of tizanidine hydrochloride synthesis. The proposed method enables selective estimation of any individual impurity among other impurities.

The mobile phase A and B programmed in linear gradient mode at a flow rate of 1.0 mL/min resolved all five peak components with resolution more than 5.0. The peak shapes of all the components were found to be symmetrical, well defined and free from tailing. The elution order was tizanidine (about 5 min), impurity-A, B, C, D and E (about 11, 12, 25, 28 and 30 min, respectively). The optimum wavelength for detection was found to be 310 nm. The linearity study of the method indicates the suitability of the method over a wide range of concentration of impurities. The high sensitivity of method allows detection and estimation of impurities even at low concentration level.

The method is specific, selective, accurate, precise and robust. It can be exclusively used as a test method for the estimation of related substances in tizanidine active pharmaceutical ingradients. A very simple method of sample preparation allows accurate determination of all five structurally

related impurities despite their different polarities and solubility characteristics. Hence it can be used for monitoring and controlling of impurities that are likely to be generated in the manufacturing of tizanidine active pharmaceutical ingradients.

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