August 2008 Volume 7 Issue 8

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 7(8) 2008 [586-590]

Identification and characterization of potential impurities of tizanidine hydrochloride

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Dr. Reddy's -IPDO-IPM Communication # 00066

Received: 15th July, 2008 ; Accepted: 20th July, 2008

ABSTRACT

During the analysis of laboratory batches of Tizanidine HCl, six impurities were detected by a simple isocratic reverse-phase high performance liquid chromatography (HPLC) whose area percentage ranged from 0.10 to 0.30 %. A thorough study has been undertaken to isolate and characterize these impurities, LCMS was performed to identify the mass number of the impurities. The structures of the impurities characterized as: N-(4,5-dihydro-1H-imidazol-2-yl)-2,1,3-benzothiadiazole-4-amine hydrochloride [imp A], N-(5-chloro-2,1,3-benzothiadiazol-4-yl)thiourea (imp B), dimer of Tizanidine HCl (imp C), S-methyl-N-(5-chloro-2,1,3-benzothiadiazol-4-yl)-isothiouronium iodide (imp D), 4-amino-5-chloro-2,1,3-benzothiadiazole (imp E), and N,N-bis(5-chloro-2,1,3-benzothiadiazol-4-yl)-N-(4,5-dihydro-1H-imidazol-2-yl)guanidine (imp F).

KEYWORDS

Tizanidie;
Drug;
Impurities;
Synthesis;
HPLC-LEMS.

1. INTRODUCTION

Tizanidine hydrochloride is a centrally acting α_2-adrenergic agonist and centrally active myotonolytic skeletal muscle relaxant[1-2]. The effects of Tizanidine are greatest on polysynaptic pathways. The overall effect of these actions is thought to reduce facilitation of spinal motor neurons. In the literature, a radioimmunoassay method for quantification of Tizanidine hydrochloride has been widely used[3]. Also, determination of Tizanidine in human plasma by Gas Chromatography-Mass spectrometry has been reporated[4]. Tizanidine, which contains a cyclic guanidine moiety, can exist as two tautomers[5]. Raman and Patil reported[6] analytical methods for estimation of Tizanidine in combination of Nimesulide and Tizanidine. Qi et al. have reported[7] stability indicating HPLC method for Tizanidine. Recently, Mahadik et. al. developed stability indicating HPTLC determination of Tizanidine hydrochloride in bulk drug and pharmaceutical formulations[8]. Tizanidine is an off-white to light yellow, fine crystalline powder, odorless or with a faint characteristic odor and slightly soluble in water and methanol, solubility in water decreases as the pH increases. Its chemical name is 5-chloro-4-(2-imidazolin-2-ylamino)-2,1,3-benzothio diazole hydrochloride. Literature studies reveal different methods for the preparation of Tizanidine hydrochloride[9].

During the analysis of laboratory batches of Tizanidine hydrochloride, six impurities were detected

whose area percentage ranged from 0.10 to 0.30 %, by a simple isocratic reverse phase LC method. A comprehensive study has been undertaken to isolate and characterize these impurities by spectroscopic and spectrometric techniques. The impurity profile study has to be carried out for any final product to identify and characterize all the unknown impurities that are present at a level of even below 0.05%. The requirement of identifying and characterizing the impurities in the final product is extremely necessary in the wake of stringent purity requirements from the regulatory authorities. This paper not only describes the isolation and characterization of six impurities that are present in the range of 0.10 to 0.30 %, in the bulk drug of Tizanidine hydrochloride but also explains the formation of these impurities.

2. EXPERIMENTAL

2.1 Samples

The investigated samples of Tizanidine hydrochloride bulk material were obtained from Dr. Reddy's Laboratories Ltd., Active Pharmaceutical Ingredient-III, Hyderabad, India.

2.2 High performance liquid chromatography (Analytical)

An in-house LC method was developed for the analysis of Tizanidine hydrochloride and its impurities, where a column (Symmetry C_{18} 250×4.6 mm, 5µ) with a mobile phase consisting of A: buffer (2.25 g of sodium perchlorate 300 ml of water, allow to dissolve and add 0.5ml of TEA, adjust pH to 3.6 with phosphorius acid then add acetonitrile and degas mobile phase B dissolve 2.0 g of sodium per chlorate 300 ml of water, allow to dissolve and add 0.5 ml of TEA, adjust pH to 2.6 with phosphoric acid then add 700 ml of acetonitrile with a timed gradient program of T/%B: 95/05, 80/20, 50/50, 35/65, 25/75, 95/05, 95/05, with a flow rate of 1.0 ml/min, UV detection at 230 nm was used. This LC method was able to detect all these impurities.

2.3 Liquid chromatography-mass spectrometry (LC-MS)

LC-MS/MS compatible method was developed for the analysis of Tizanidine hydrochloride and its impurities, where a column (Inertsil ODS 3V 250X4.6X5.0u) with a mobile phase consisting of 0.01M ammonium acetate (pH = 6.0) adjusted with dilute acetic acid and acetonitrile in the ratio of 65:35, with a flow rate of 1.0 ml/min, UV detection at 230 nm was used. This LC method was able to detect all the impurities. The mass spectrum of impurities were recorded on AB-4000 Q-trap LC-MS/MS mass spectrometer.

2.4 Mass spectrometry

The electro spray ionization and MS-MS studies were performed on AB-4000 LC-MS/MS mass spectrometer. The positive and negative electro spray MS data was obtained by switching the capillary voltage between n+5000 and - 4500V respectively.

2.5 NMR spectroscopy

The ^{1}H-NMR done at 400 MHz and 100 MHz on Varian Mercury plus 400 MHz FT NMR Spectrometer and similar experiments for impurities A,B,C,D,E and F were performed on Gemini-2000 (200 MHz) in DMSO-d_6. The ^{1}H chemical shift values were reported on the δ scale in ppm, relative to TMS (δ=0.00 ppm) and the chemical shift values were reported relative to $CDCl_3$ (δ=77.00 ppm) and DMSO-d_6 (δ=39.50 ppm) as internal standards respectively.

2.6 FT IR spectroscopy

The IR spectra were recorded on the solid state as KBr dispersion medium using Perkin Elmer spectrum one FT IR spectrophotometer.

2.7 Synthesis of impurities

The impurity A is prepared by taking deschloro compound of the starting material and further reaction same as steps used for the preparation of Tizanidine, Impurity B is one of the key intermediate in the preparation of Tizanidine impurity C was prepared by taking less molar equivalent of ethylene diamine with respective S-methyl-N-(5-chloro-2,1,3-benzothiadiazol-4-yl)-isothiouronium iodide, impurity D is one of the key intermediate in the synthesis of Tizanidine, impurity E is formed during condensation of ethylene diamine and S-methyl-N-(5-chloro-2,1,3-benzothiadiazol-4-yl)-isothiouronium iodide and impurity F is formed due to condensation of Tizanidine and S-methyl-N-(5-chloro-2,1,3-benzothiadiazol-4-yl)-isothiouronium iodide.

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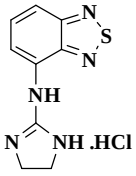
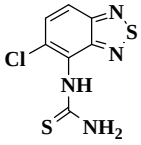
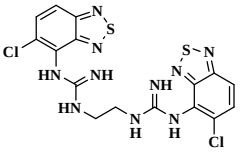
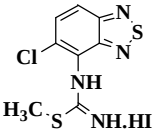
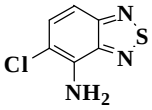
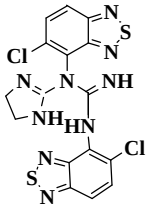
3. RESULTS AND DISCUSSIONS

3.1 Detection of impurities A, B, C, D, E and F

A typical analytical LC chromatogram of a laboratory batch of Tizanidine Hydrochloride bulk drug recorded using the LC method as described in section 2.2. The LC-MS compatible method is described in section 2.3 which is used to detect the impurities (figure 1). Retention times in HPLC and structures of these impurities and Tizanidine are shown in TABLE 1. Impurities B, C, D, E and F are polar and impurity A is non-polar respectively with respect to tizanidine hydrochloride.

TABLE 1: Retention time from HPLC, molecular weight of the impurities from LC-MS

S. no	Ret. time* min	Compound	M.W	Structure	Nature
01	0.88	Impurity-A	255.5		Process related
02	1.51	Impurity-B	244.5		Process related
03	1.65	Impurity-C	480.4		Process related
04	2.24	Impurity-D	386.7		Process related
05	3.11	Impurity-E	185.6		Process related
06	3.15	Impurity-F	464.4		Process related

*Retention time of impurities in HPLC

3.2 Structural elucidation of tizanidine hydrochloride impurities

Structural elucidation of impurities

Structural elucidation of impurity A in ES-MS spectrum displayed molecular ion at m/z 219 (M-H). The ES-MS-MS spectrum displayed daughter ions at m/z 190.11, 135.0 and 162.0 in which 219 is the dominant fragment. From the mass spectral data the molecular ion of impurity is 219, the exchangeable proton were identified by D_2O ^{1}H-NMR spectra. IR spectrum displayed characteristic absorptions at 3269, 3140 and 2985 cm^{-1} corresponding to NH, ArCH, alphatic-CH repectively, the impurity A could be $C_9H_9N_5S.HCl$. This molecular formula matched well with the molecular ion observed at m/z 219.0 in the mass spectral data. The data obtained form the spectral studies can be rationalized in terms of impurity A having the molecular formula $C_9H_9N_5S.HCl$, and the corresponding structure was characterized as N-(4,5-dihydro-1H-imidazol-2-yl)-2,1,3-benzothiadiazole-4-amine hydrochloride [impurity A]. The above mentioned strategies were used for elucidation of the impurities from B to F[5](Figure 2), TABLES (1-3).

3.3 Formation of impurities

The Impurity A is formed due to presence des chloro compound in the starting material and further reaction forms the impurity, Impurity B is one of the key intermediate in the preparation of Tizanidine; impurity C was formed during condensation of ethylene diamine with

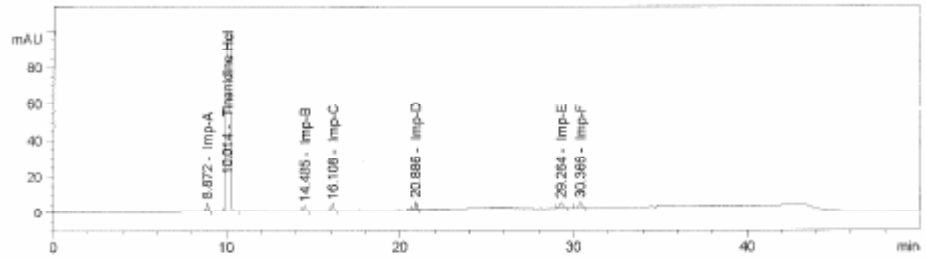


Figure 1: LC method used for LC-MS

TABLE 2: FT-IR and mass spectral data of tizanidine hydrochloride impurities A, B, C, D, E and F

S. no.	Compound	IR*	MS
1.	Impurity-A	3269 (N-H stretching), 3140 (Aromatic C-H stretching), 2985, 2853 (Aliphatic C-H stretching), 2853 (prorogated NH stretching) 1647,1618 (Aromatic C=C stretching), 1542 (-N-H bending), 1484 (aliphatic C-H bending), 1272 (aromatic C-H stretching), 1292 (C-N stretching). 757 (aromatic C-H bending)	+veES-MS:219.0(M+H)
2.	Impurity-B	3340 (N-H stretching), 3137 (Aromatic C-H stretching), 2775, 2669 (Aliphatic C-H stretching), 1638.05(Aromatic C=C stretching), 1582 (-N-H bending), 1471.86 (aliphatic C-H bending), 1275.44 (aromatic C-H stretching), 752.56 (aromatic C-H bending)	+veEIMS:245 $(M+H)^+$
3.	Impurity-C	3248 (N-H stretching), 3122 (Aromatic C-H stretching), 2992 (Aliphatic C-H stretching), 1667,1639 (Aromatic C=C stretching), 1593 (-N-H bending), 1488.4 (aliphatic C-H bending), 1261 (aromatic C-H stretching), 770 (aromatic C-H bending)	+veEIMS:481$(M+H)^+$
4.	Impurity-D	34.37.9 (N-H stretching), 3335.4 (Aromatic C-H stretching), 2921.6 (Aliphatic C-H stretching), 1636.2 (Aromatic C=C stretching), 1584 (-N-H bending),	+veEIMS:387.7$(M+H)^+$
5.	Impurity-E	3495,3389 (primary N-H stretching), 1606 (primary N-H stretching), 1575,1486 (aromatic C=C stretching), 1537 (C=N stretching), 1348 (aromatic C-N stretching), 1107 (aromatic C-Cl stretching), 836 (aromatic C-H bending), 770 (aromatic C-H bending)	+veEIMS:186.7$(M+H)^+$, 187.6(M+2H)
6.	Impurity F	3248 (N-H stretching), 3122 (Aromatic C-H stretching), 2992 (Aliphatic C-H stretching), 1667,1639 (Aromatic C=C stretching), 1593 (-N-H bending), 1488.4 (aliphatic C-H bending), 1261 (aromatic C-H stretching), 770 (aromatic C-H bending)	+veEIMS:465(M+H)

***KBr (Impurities A,B,C,D,E, and F and tizanidine hydrochloride)**

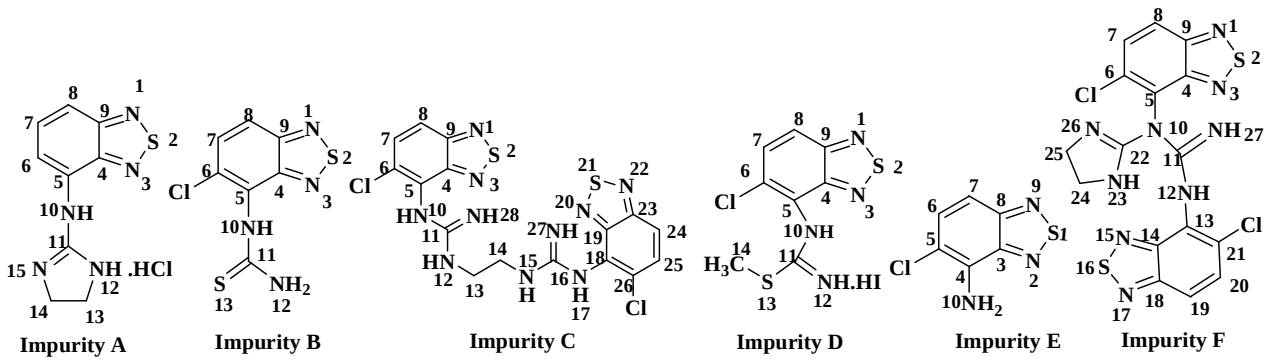


Figure 2

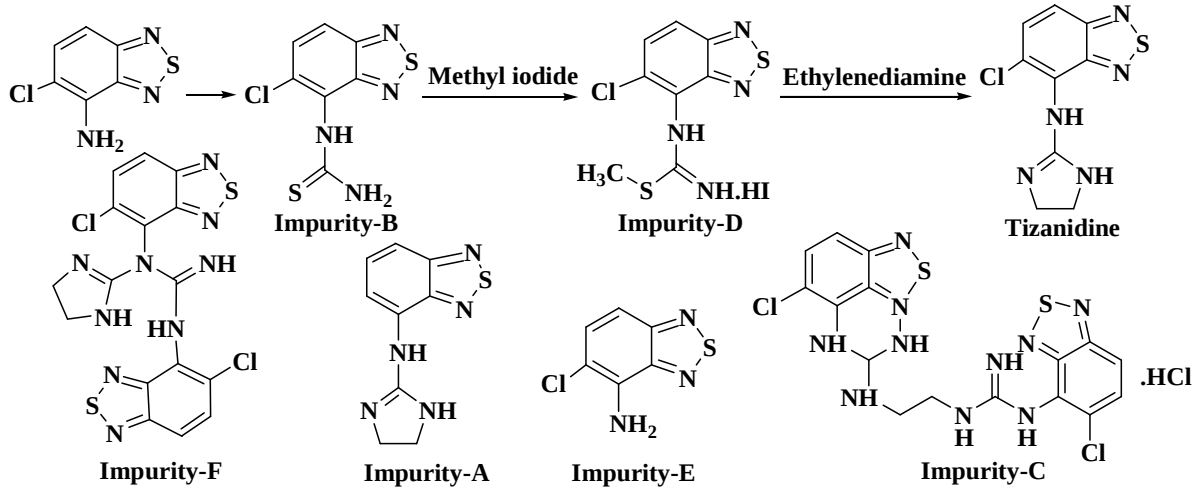


Figure 3: Synthesis of impurities

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TABLE 3: ^{1}H assignments for tizandine hydrochloride impurities A, B, C, D, E and F

Position*	Impurity-A		Impurity-B		Impurity-C		Impurity-D		Impurity-E		Impurity-F	
	^{1}H	ppm / J	^{1}H	ppm / J	^{1}H	ppm / J	^{1}H	ppm / J	^{1}H	ppm / J	^{1}H	ppm / J
1												
2												
3												
4												
5												
6	1H	7.6/d							1H	7.45/d		
7	1H	7.78/dd	1H	7.8/d	1H	8.1/d	1H	7.68/d	1H	7.28/d	1H	7.8/s
8	1H	8.07/d	1H	7.6/d	1H	7.9/d	1H	6.82/d	-	-	1H	7.6/s
9	-	-			-	-	-	-	-	-		
10	1H	11.33/br-s	NH	7.1	NH	3.5	NH	3.49/ br-s	NH_2	5.01/ s		
11	-	-										
12	1H	8.54/br-s	NH	2.8	NH	2.4	NH	2.5/ s			1NH	2.5/s
13	2H	3.7/s										
14	2H	3.7/s					3H	2.43/s				
15	NH	8.54/ br-s										
16												
17												
18												
19											1H	7.6/s
20									3H	3.84/s	1H	7.8/s
21												
22												
23											1NH	
24											2H	3.4/t
25											2H	4.2/t
26												
27											NH	7.0/s

*S- Singlet, d- Doublet, m- Multiplet, br- broad

Impurity D, where ethylene diamine molar ratio is less, impurity D is one of the key intermediate in the synthesis of Tizanidine, impurity E is formed during condensation of ethylene diamine and impurity D is an intermediate and finally impurity F is formed due to condensation of Tizanidine and impurity D are shown in figure 3.

ACKNOWLEDGMENTS

The authors thank the management of Dr. Reddy's Laboratories Ltd., IPDO Bachupally, for supporting this work. Co-operation from colleagues of analytical R&D, Dr. Reddy's Laboratories Ltd., IPDO, Bachupally, is gratefully acknowledged.

REFERENCES

[1] P.Koch, D.R.Hirst, B.R.Von Wartburg; Xenobiotica, **19**, 1255-1260 **(1989)**.
[2] F.L.S.Tse, J.M.Jaffe, S.Bhuta; Fundam.Clin. Pharmacol., **1**, 479-485 **(1987)**.
[3] V.Healzlewood, P.Symoniw, P.Maruff, M.J.Eadie; Eur.J.Clin.Pharmcol., **25**, 65-72 **(1983)**.
[4] J.Lee, J.H.Seo, D.H.Kim; Analyst, **127**, 917-920 **(2002)**.
[5] L.J.Jackman, T.Jen; J.Am.Chem.Soc., **97**, 2818 **(2002)**.
[6] B.Raman, D.Patil; Indain Drugs, **39**, 392-394 **(2002)**.
[7] M.L.Qi, P.Wang, L.Wang; Anal.Chim.Acta, **478**, 171-177 **(2003)**.
[8] K.R.Mahadik, A.r.Paradkar, H.Agrawal, K.Neeraj; J.Phram.Biomed.Anal., **3**, 545-552 **(2003)**.
[9] W.T.Smith,JR, W.Y.Chen; J.Org.Chem., **27**, 676-677 **(1962)**; M.Weinstock, P.Davis, B.Handelsman, R.Tull; J.Org.Chem., **32**, 2823-2829 **(1967)**.