



Analytical Characterization of Two New Related Impurities of Diltiazem by High Resolution Mass Spectrometry and NMR techniques

JAGADEESH NARKEDIMILLI^{1,2,*}, Y. RAVINDRAKUMAR¹,
SANDEEP MOHANTY¹, T. SRINIVASARAO¹ and A. JAYASHREE²

¹Dr. Reddy's Laboratories Limited, API Plant-III, Medak District, Hyderabad - 502325,, India.

²Centre for chemical sciences & Technology, Institute of Science and Technology, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad 500085, India.

*Corresponding author E-mail: jagadeeshn@drreddys.com, nark_012@yahoo.com

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ABSTRACT

Diltiazem (DTZ) is an optically active calcium channel blocker having a benzodiazepine structure. Two impurities (referred as DTZ-I and DTZ-II) were detected with area percentages ranging from 0.1% to 0.15% during the impurity profile study of Diltiazem hydrochloride drug substance. A simple isocratic high performance liquid chromatographic method (HPLC) and liquid chromatography–mass spectrometry (LC–MS) were used for the detection. The impurities were isolated by preparative column chromatography. Analytical information from nuclear magnetic resonance and mass spectral data of the impurities revealed their structures as 2-(4-methoxyphenyl)-5-methyl-4-oxo-2,3,4,5-tetrahydrobenzo[b][1,4]thiazepin-3-yl acetate (DTZ-I) and 2-(4-methoxyphenyl)-4-oxo-5-vinyl-2,3,4,5-tetrahydrobenzo[b][1,4]thiazepin-3-yl acetate (DTZ-II). Impurity identification, isolation and structure elucidation were discussed.

Key words: Diltiazem; Impurities; Isolation; characterization techniques.

INTRODUCTION

Diltiazem (DTZ), (2S, 3S)-3-acetyloxy-5-[2-(dimethyl-amino)ethyl]-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepin-4(5H)-one, is a benzothiazepine calcium-channel blocker with peripheral and coronary vasodilator properties. It is widely used in the management of angina

pectoris and hypertension. Treatment with diltiazem is generally well tolerated; however, a variety of skin disorders have been associated with diltiazem therapy; these cutaneous adverse reactions include acne, rash, urticaria and dermatitis¹.

During the analysis of different laboratory batches of Diltiazem drug substance, two unknown

impurities with area percentages ranging from 0.1% to 0.15% were detected by a simple isocratic HPLC method. To commercialize an active pharmaceutical ingredient (API), as per regulatory requirement, it is mandatory for the manufacturer to identify and characterize all the unknown impurities that are present in API at a level of even below 0.05%². In this context, a comprehensive study has been undertaken to identify and characterize two impurities present in the laboratory batches of Diltiazem hydrochloride drug substance using spectroscopic and spectrometric techniques.

During the literature survey of Diltiazem³⁻²⁷ no reports found to unknown impurities isolation and characterization. The study toward the identification and characterization of impurities in Diltiazem was not reported in the literature to date, to the best of our knowledge, and this impurity profiling study will be immense importance for process development chemist to understand the source of impurities during the synthesis of Diltiazem hydrochloride (Scheme 1)

EXPERIMENTAL

Samples and reagents

The investigated samples of Diltiazem hydrochloride were obtained from synthetic R&D laboratory of Dr. Reddy's Laboratories Ltd., CTO-VI, pydibhimavaram, India. Reagents used for analysis, i.e., d-10-camphorsulfonic acid, sodium acetate, sodium hydroxide and acetic acid (AR grade), methanol and acetonitrile (HPLC grade) were obtained from Merck (india) Limited. Dimethylsulphoxide-d₆ was purchased from Aldrich Chemical Co., USA. Milli-Q grade water was used.

High performance liquid chromatography (Analytical)

A Waters Model Alliance 2695-separation module (Waters corporation, Milford, MA, USA) equipped with a waters 2998-photo diode array UV detector was used. Data was processed through Waters empower software. The analysis was carried out on Inertsil C18 column, 250 x 4.6mm i.d., 5µm particle size (GL Sciences Inc., Japan) with a mobile phase consisting of Isocratic mixture of buffer, acetonitrile and methanol in the ratio of 50: 25: 25 (v/v/v) dissolved 1.16 gr of d-10-camphorsulfonic

acid in 1000 ml of 0.1 M sodium acetate pH 6.2 adjusted with sodium hydroxide as buffer. The column temperature was maintained at ambient condition. Flow rate was kept at 1.6 mL min⁻¹ and the column eluent was monitored at 240 nm.

Preparative liquid chromatography

An Agilent 1200 preparative chromatography system equipped with Agilent G1315D photo diode array UV detector, Fraction collector model Agilent G1346B and Rheodyne Injector Model 2260A with 1.8 ml loop was used. A 250 x 20 mm i.d column packed with 10µm YMC Actus triart C18 (YMC Co. Ltd., Japan) was employed for separation with a mobile phase consisting of 30 volumes of 0.1% acetic acid (Aq) and 70 volumes of Acetonitrile was used with UV detection at 240 nm at a flow rate of 20.0 mL min⁻¹. The column temperature was maintained at ambient temperature. The data was recorded using Agilent Chemstation software.

Mass spectrometry

The High Resolution Mass Spectra were obtained from a Waters LCT Premier time of- flight (TOF) mass spectrometer (Milford, USA) with ESI source. Resolution of the LC-TOF/MS was more than 5000. Leucine enkephalin (C₂₈H₃₇N₅O₇) was used as external lock-mass. The source block and desolvation temperatures were 90°C and 180°C respectively. The nebulizer and desolvation gas (nitrogen) flows were 20 L h⁻¹ and 450 L h⁻¹, respectively. The instrument parameters were used as capillary 3000 V, cone 25 V, extractor 2 V and MCP 2700 V. The acquisitions were done in scan mode. Data acquisition and processing were done using Mass Lynx V.4.0 software. Inertsil C18 column (4.6 mm x 250 mm, 5 µm) with a mobile phase consisting of 30 volumes of 0.1% acetic acid (Aq) and 70 volumes of Acetonitrile was used for the separations. The column temperature was maintained at 30°C. Flow rate was kept at 1.0 mL min⁻¹ and the column eluent was monitored at 240 nm. The diltiazem sample was prepared in methanol at 1.0 mg ml⁻¹ concentration and 10 µL of sample solution was injected in LCMS system.

Nuclear magnetic resonance spectroscopy

¹H, ¹³C, NMR and two dimensional (2D) NMR experiments such as gradient Double

Quantum Filtered Correlation Spectroscopy (gDQCOSY), gradient Heteronuclear Single Quantum Coherence Spectroscopy (gHSQC) and gradient Heteronuclear Multibond Coherence Spectroscopy (gHMBC) were performed on 500 MHz Varian Unity Inova FT-NMR instrument at 25°C in DMSO-d₆. The ¹H and ¹³C chemical shift values were reported on the δ scale in ppm, relative to TMS (δ = 0.00 ppm).

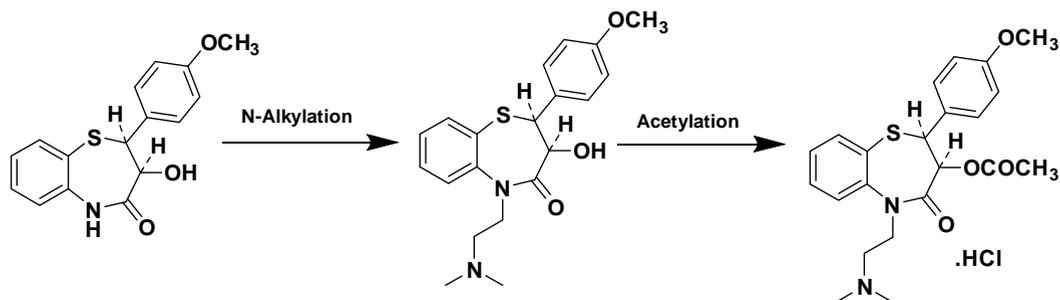
RESULTS AND DISCUSSION

Detection of impurities

Diltiazem hydrochloride samples were analyzed by the HPLC method described. The unknown impurities observed at levels of 0.1 to 0.15%. These samples were subjected to LC-HRMS analysis. Isolated these unknown impurities by Preparative HPLC. Isolated two impurities were

Table 1: NMR assignments of DTZ, DTZ-I and DTZ-II, ^aRefers structural formula in Fig. 2 for numbering

Position ^a	DTZ			DTZ-I			DTZ-II		
	¹ H	ppm	¹³ C	¹ H	ppm	¹³ C	¹ H	ppm	¹³ C
1	-	-	169.23	-	-	169.146	-	-	169.238
2	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-
4	3	1.83	20.205	3	1.83	20.267	3	1.84	20.167
5	-	-	144.344	-	-	146.223	-	-	140.808
6	-	-	-	-	-	-	-	-	-
7	-	-	167.023	-	-	166.518	-	-	165.121
8	1	5.17	53.112	1	5.15	53.425	1	5.11	52.233
9	1	4.99	70.42	1	5.07	70.359	1	5.02	70.665
10	-	-	-	-	-	-	-	-	-
11	-	-	127.99	-	-	127.288	-	-	128.159
12	1	7.72	135.3	1	7.69	134.857	1	7.82	135.721
13	1	7.4	126.593	1	7.37	126.547	1	7.51	129.174
14	1	7.7	131.573	1	7.6	131.16	1	7.65	131.0
15	1	7.7	124.591	1	7.6	124.294	1	7.55	127.0
16	-	-	-	-	-	-	-	-	-
17	-	-	127.227	-	-	126.913	-	-	126.516
18	1	7.4	130.771	1	7.37	130.756	1	7.33	130.702
19	1	6.9	113.554	1	6.93	113.485	1	6.93	113.546
20	-	-	159.201	-	-	159.178	-	-	159.262
21	1	6.9	113.554	1	6.93	113.485	1	6.93	113.546
22	1	7.4	130.771	1	7.37	130.756	1	7.33	130.702
23	-	-	-	-	-	-	-	-	-
24	3	3.77	55.044	3	3.77	50.044	3	3.76	55.044
25	Ha	4.3	43.648	3	3.38	35.872	1	7.55	131.741
	Hb	4.5							
26	Ha	3.1	52.355	-	-	-	Ha	4.38	99.14
	Hb	3.5					Hb	4.71	
27	-	-	-	-	-	-	-	-	-
28	3	2.78	41.998	-	-	-	-	-	-
29	3	2.78	41.998	-	-	-	-	-	-



Scheme 1: Synthetic application of Diltiazem Hydrochloride

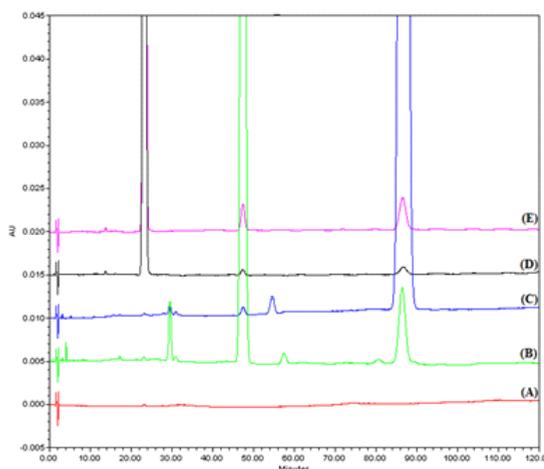


Fig. 1: Typical HPLC overlay chromatogram of blank (A), DTZ-I (B), DTZ-II (C), Test sample of Diltiazem (D), DTZ-I and DTZ-II spiked to test sample of Diltiazem (E)

coinjecting with diltiazem to confirm the retention times (Figure 1). The chemical structures of these two impurities were shown in Figure 2.

Structure elucidation of DTZ-I impurity

In the positive mass spectra, the protonated $[M+H]^+$ molecular ion was detected at m/z 358.1. The even m/z number of $[M+H]^+$ ions suggest that DTZ-I contains odd number of nitrogen atoms (nitrogen rule). From these results the molecular ion of DTZ-I was found to be at m/z 357. The mass difference between Diltiazem and DTZ-I was found to be 57 amu less. The positive HR-MS spectrum showed protonated molecular ion at m/z 358.1102 corresponding to molecular formula $C_{19}H_{20}NO_4S$. When compared with the molecular formula of Diltiazem, there was a difference of C_3H_7N . The difference can be rationalized in terms of the

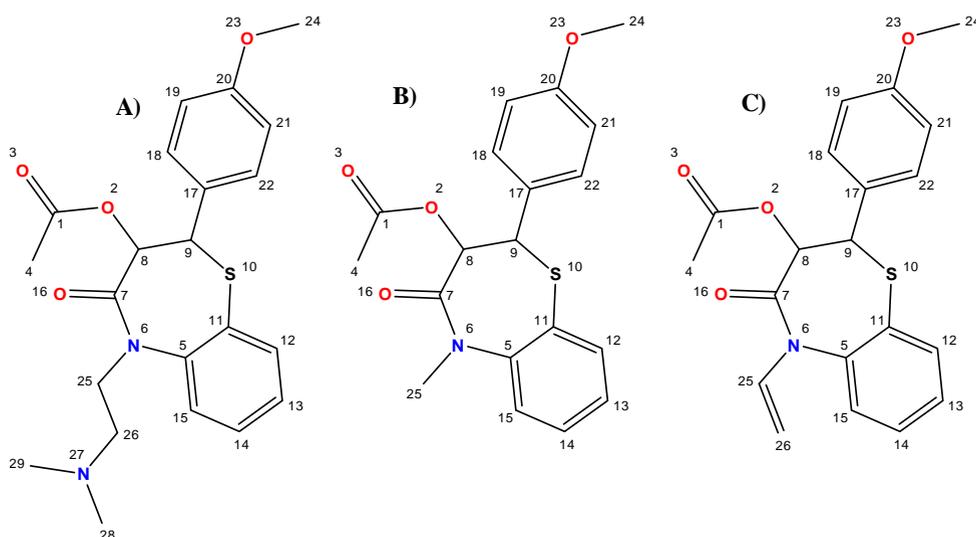


Fig. 2: Chemical Structure of DTZ (A), DTZ-I (B), DTZ-II (C), Atom numbering used for NMR assignments

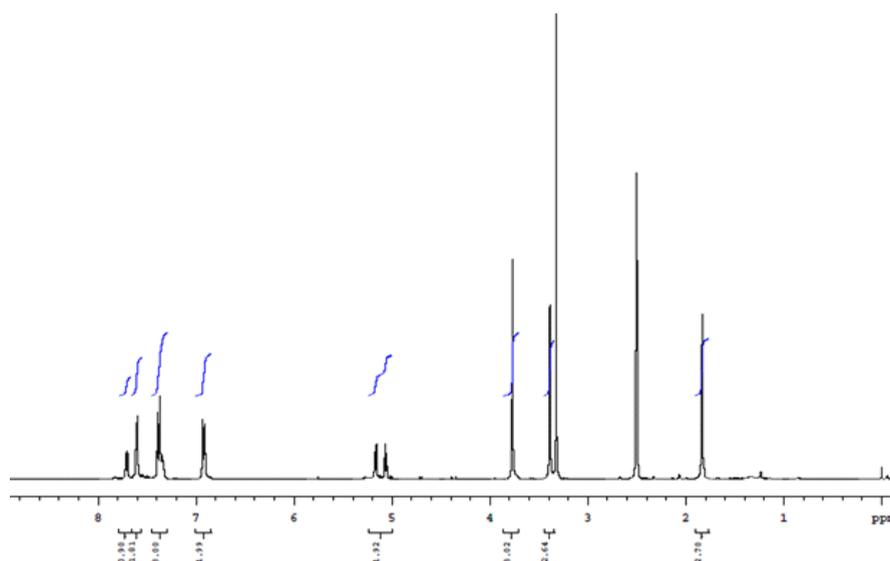


Fig. 3: ¹H NMR spectrum of DTZ-I

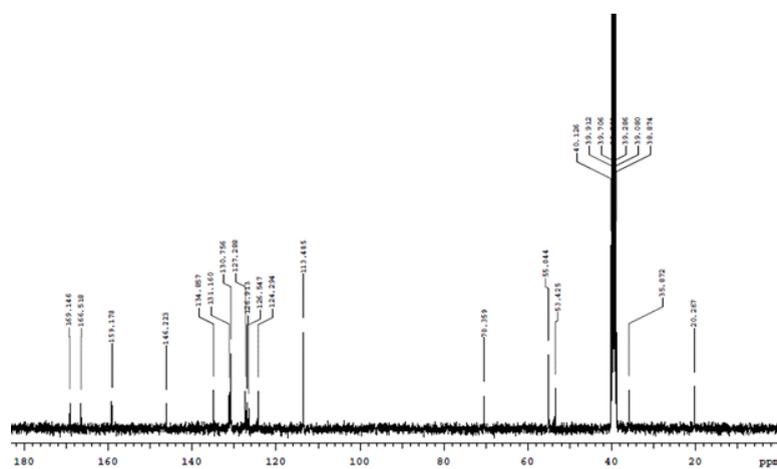


Fig. 4: ¹³C NMR spectrum of DTZ-I

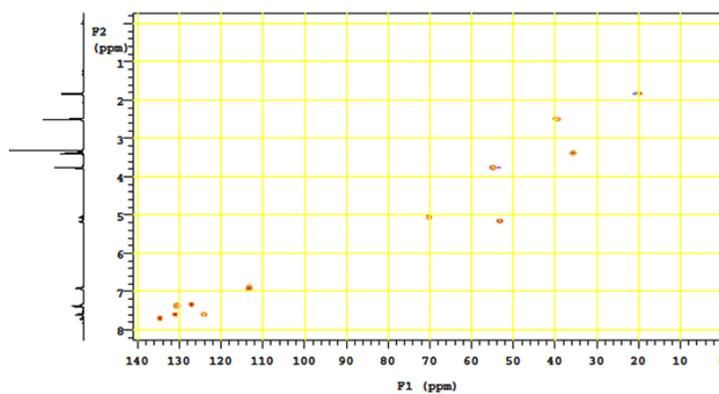


Fig. 5: HSQC NMR spectrum of DTZ-I

loss of N, N-dimethylethanamine moiety. The presence of additional methyl signal at δ 3.38 ppm in ^1H NMR (Figure 3) and corresponding carbon signal at 35.872 ppm in ^{13}C NMR (Figure 4), The correlation between the carbon and proton clearly observed in HSQC (Figure 5) and no methylene protons were observed in HSQC. The methyl proton at δ 3.38 ppm showed HMBC (Figure 6) correlations for carbon C5 and C7 positions at 146.223 and

166.518 ppm respectively (Figure 7). The methyl proton at δ 3.38 ppm didn't show any correlation in COSY (Figure 8), which confirms the N-Methyl in the structure. NMR assignments are shown in Table 1. The above spectral data supports the assigned structure as 2-(4-methoxyphenyl)-5-methyl-4-oxo-2,3,4,5-tetrahydrobenzo[b][1,4] thiazepin-3-yl acetate (DTZ-I).

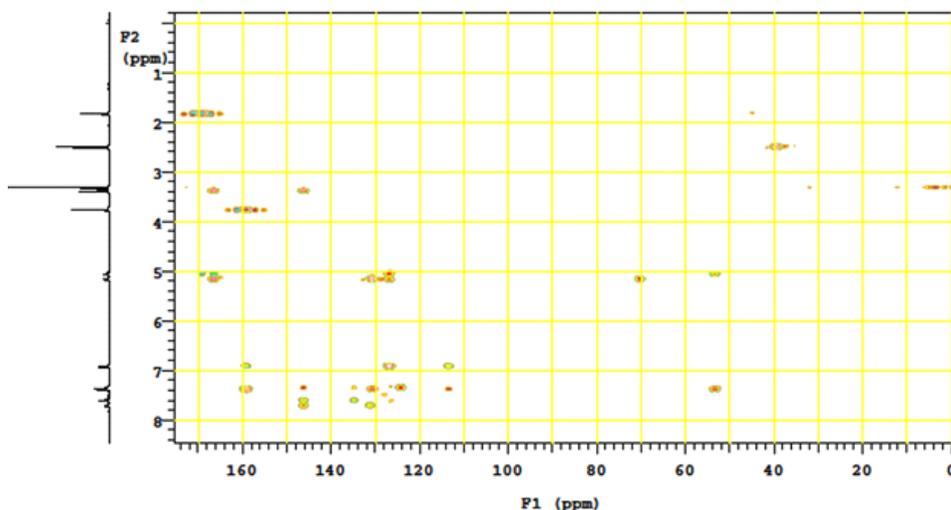


Fig. 6: HMBC NMR spectrum of DTZ-I

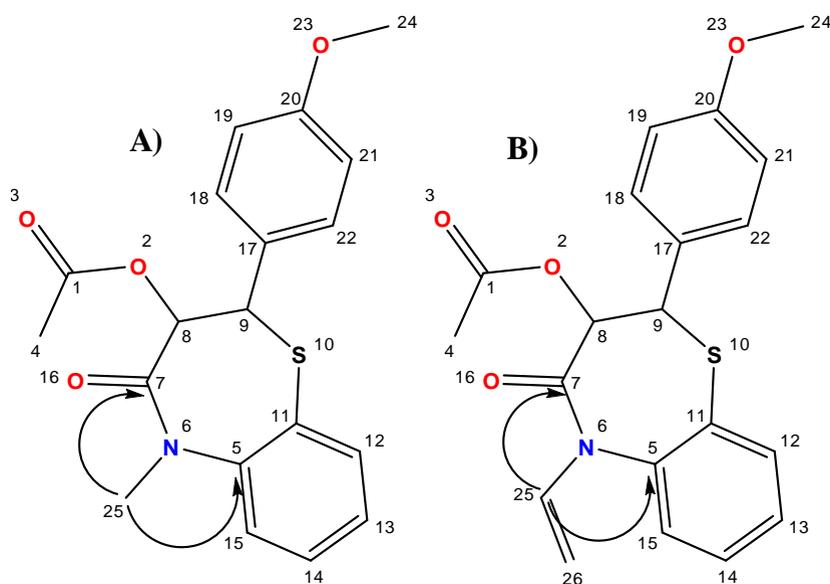


Fig. 7: HMBC linkage of C5 and C7 carbons to C25 methyl proton in DTZ-I (A), HMBC linkage of C5 and C7 carbons to C25 methine proton in DTZ-II (B)

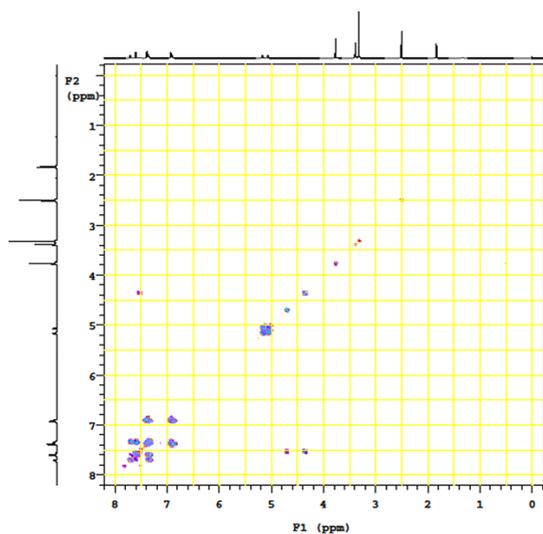


Fig. 8: COSY NMR spectrum of DTZ-I

Structure elucidation of DTZ-II impurity

In the positive mass spectra, the protonated $[M+H]^+$ molecular ion was detected at m/z 370.1. The even m/z number of $[M+H]^+$ ions suggest that DTZ-II contains odd number of nitrogen atoms (nitrogen rule). From these results the molecular ion of DTZ-II was found to be at m/z 369. The mass difference between Diltiazem and DTZ-II was found to be 45 amu less. The positive HR-MS spectrum showed protonated molecular ion at m/z 370.1131 corresponding to molecular formula $C_{20}H_{20}NO_4S$. When compared with the molecular formula of Diltiazem, there was a difference of C_2H_7N . The difference can be rationalized in terms of the loss of trimethyl amine moiety. The presence of additional aromatic signal at δ 7.55 ppm in 1H NMR (Figure 9) and corresponding carbon signal

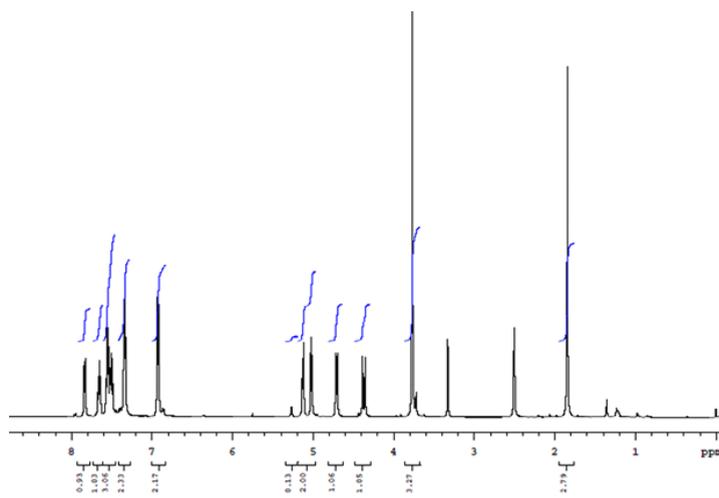


Fig. 9: 1H NMR spectrum of DTZ-II

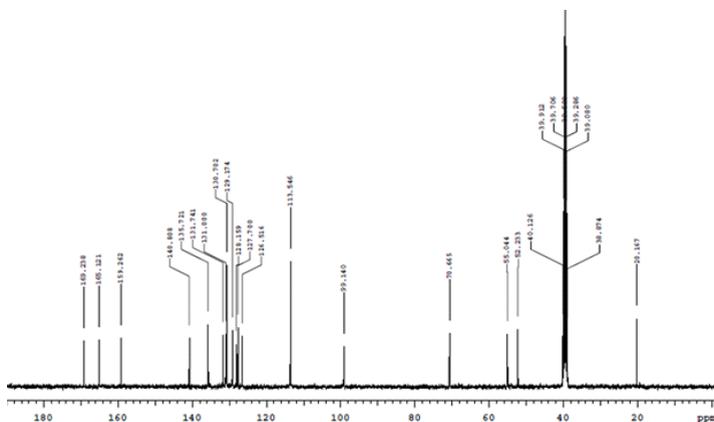


Fig. 10: ^{13}C NMR Spectrum of DTZ-II

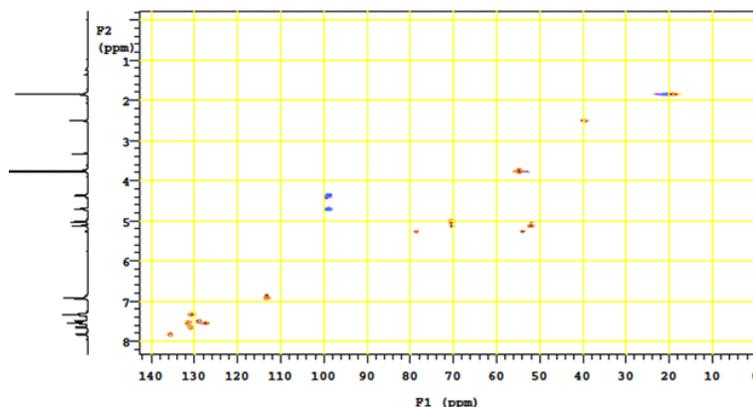


Fig. 11: HSQC NMR Spectrum of DTZ-II

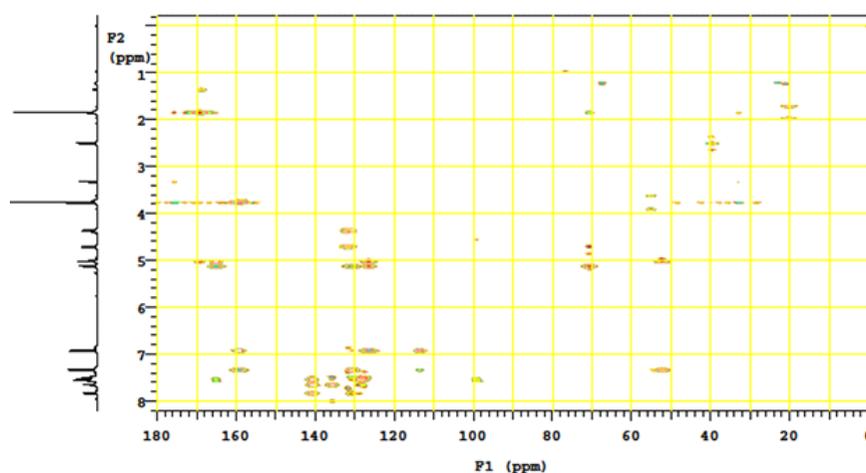


Fig. 12: HMBC NMR Spectrum of DTZ-II

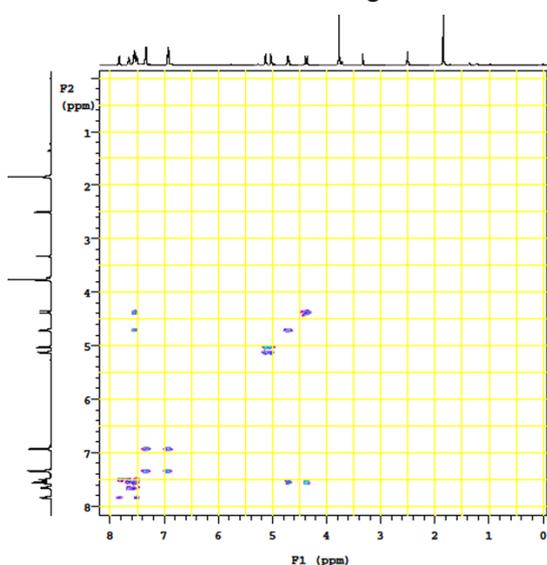


Fig. 13: COSY NMR Spectrum of DTZ-II

at 131.741 ppm in ^{13}C NMR (Figure 10). The correlation between the carbon and proton clearly observed in HSQC (Figure 11) and one methylene signal was observed in HSQC. The methylene proton at δ 4.38 and 4.71 ppm in ^1H NMR corresponding carbon signal at 99.41 ppm in ^{13}C NMR. The aromatic methine proton at δ 7.55 ppm showed HMBC (Figure 12) correlations for carbon C5 and C7 positions at 140.808 and 165.121 ppm respectively (Figure 7). The aromatic methine proton at δ 7.55 ppm showed correlation with methylene proton at δ 4.38 and 4.71 ppm in COSY (Figure 13). NMR assignments are shown in Table 1. The above spectral data supports the assigned structure as 2-(4-methoxyphenyl)-4-oxo-5-vinyl-2,3,4,5-tetrahydrobenzo[b][1,4]thiazepin-3-yl acetate (DTZ-II).

CONCLUSION

The related impurities in Diltiazem hydrochloride were identified, isolated and characterized by HPLC (analytical and preparative), LC-MS and NMR (¹H, ¹³C and 2D) techniques.

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