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Identification and characterization of impurity in olmesartan medoxomil bulk drug

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ABSTRACT

Olmesartan medoxomil is an angiotensin II antagonist and is used as antihypertensive agent. Impurity profiling of olmesartan medoxomil drug substance by reverse phase HPLC reveals the presence of a potential impurity. It was isolated by preparative liquid chromatography(Prep LC) and structural characterization was done by NMR and FT-IR. Further characterization was done on the basis of fragmentation pattern of this impurity by LC-MS/MS using Electron Spray Ionization (ESI) source and triple quadrapole mass analyzer. This impurity is characterized as 5-methyl-2-oxo-[1,3]dioxo-4-yl methyl-4-(1-methoxy-1-methyl-ethyl)-2-propyl-1-{4-[2'(1H-tetrazol-5-yl)phenyl] phenyl}methyl imidazole-5-carboxylate.

KEYWORDS

Olmesartan;
Impurity;
Isolation;
Characterization;
LC-MS/MS;
NMR.

INTRODUCTION

Olmesartan medoxomil chemically known as (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 5-(1-hydroxy-1-methyl-ethyl)-2-propyl-3-[[4-[2-(2H-tetrazol-5-yl) phenyl]phenyl]methyl]-3H-imidazole-4-carboxylate (Figure 1) a specific angiotensin II antagonist, is used alone or with other antihypertensive agents to treat hypertension. Olmesartan medoxomil is rapidly and completely bioactivated by ester hydrolysis to olmesartan during absorption from the gastrointestinal tract.

Analysis of olmesartan medoxomil in plasma and urine samples by LC-MS/MS technique and HPLC techniques has been reported. During the HPLC analysis of olmesartan medoxomil a potential impurity was detected. In view of the stringent quality requirements of global regulatory authorities it is mandatory to know the structural details of potential impurity appearing above 0.1% in the Active Pharmaceutical Ingredient (API). A thorough investigation was under taken to identify and characterize the impurity.

Accordingly, this impurity was isolated by preparative HPLC and structure was confirmed by using vari-

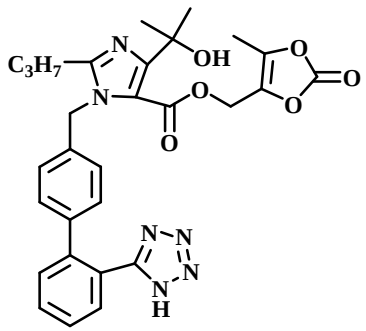

Figure 1: Chemical structure of olmesartan medoxomil

ous analytical techniques. In this paper we describe the identification and characterization of this impurity.

EXPERIMENAL

Materials

Olmesartan medoxomil was synthesized in process chemistry department of Advinus Therapeutic Research Centre, India. Ammonium acetate and sodium-dihydrogen ortho-phosphate, AR grade was obtained from Rankem, India. Methanol, acetonitrile (HPLC grade) and trifluoro acetic acid (AR grade) were procured from Spectrochem, India. De-ionized water of 18MΩ was purified by Milli-Q water purification system (Millipore, USA).

HPLC(Analytical)

Chromatographic separation was performed in Agilent1200Series HPLC system consisting of quaternary solvent delivery module, auto sampler and UV detector. Data was processed through Chemstation software version B-02-01-SR1 (260).

An Inertsil ODS C18 column with dimentions of 250mm×4.6mm i.d packed with 5µm particle size was employed for separation. The gradient program used was mobile phase consisting of 10mM sodium dihydrogen ortho phosphate pH adjusted to 2.7 with tri-fluoro acetic acid(A) and acetonitile (B) (For gradient conditions see TABLE 1) . Flow rate was kept at 1.0ml/min and column eluent was monitored at 254nm.

HPLC(Preparative)

Preparative HPLC system used was Agilent 1200 Series HPLC system equipped with binary pump, G2260A auto sampler, G1364B fraction collector and G1315B DAD detector. Sample was injected using auto-sampler. Data was processed through Chemstation software version B-02-01(244).

An Inertsil ODS C18 column with dimentions of 250mm×20mm i.d packed with 5µm particle size was employed for separation. The gradient programme used was mobile phase consisting of 10mM ammonium acetate (A) and methanol (B) (For gradient conditions see TABLE 2). Flow rate was kept at 20.0ml/ min and column eluent was monitored at 254 nm.

NMR spectroscopy

TABLE 1 : Gradient conditions for HPLC

Time (min)	% of solvent A	% of solvent B
0	80	20
5	80	20
15	50	50
20	50	50
25	30	70
30	30	70
35	80	20
40	80	20

Solvent A: 10mM Sodium dihydrogen ortho phosphate Ph adjusted to 2.7 with Tri fluor acetic acid; Solvent B: Acetonitrile

TABLE 2 : Gradient conditions for HPLC(Preparative)

Time (min)	% of solvent A	% of solvent B
0	40	60
5	40	60
15	25	75
20	25	75
23	40	60
26	40	60

Sovent A: 10mM Ammonium acetate; Solvent B: Methanol

The ^{1}H and ^{13}C experiments were performed with Varian-400 MHz with dual broad band ^{1}H chemical shift values were reported on the δ scale in ppm relative to TMS (δ=0.000ppm) and ^{13}C chemical shifts values were reported relative to DMSO-d6 (δ=39.5ppm).

FT-IR spectroscopy

The IR spectra were recorded in the solid state as KBr dispersion using Perkin Elmer FT-IR Spectrum 100 with DRS technique.

Mass spectroscopy

LC-MS/MS studies were carried out on API-2000 (LC-MS/MS triple quadrupole system Sciex, Applied Bio-Systems, Canada) the HPLC consisted of an Agilent-1100 series quaternary gradient pump with a degasser, auto sampler and column oven. The chromatographic conditions described in TABLE 2 have been used for analysis. The HPLC effluent was introduced into electron spray ionization(ESI) source of the mass spectrometer at 1.0ml/min with split ratio of 3:7. The ion source voltage was maintained at 5500 volts and capillary temperature at 350^{0}C. Nitrogen was used as both nebulizer and turbo spray gas. Mass range was kept at 50-1000 amu. MS/MS studies were carried out by maintaining normalized collision energy at 35eV with the range m/z 50-600amu.

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RESULTS AND DISCUSSION

Detection of impurity by reverse phase HPLC

The olmesartan medoxomil samples were diluted in the concentration of 0.5mg/ ml in methanol and analysed using solvent system described in TABLE 1. The impurity eluted at 17.1minutes and olmesartan medoxomil eluted at 16.2 minutes. The typical analytical LC chromatogram is shown in figure 2. This impurity was isolated by chromatographing the crude sample of olmesartan medoxomil on preparative LC.

Isolation of impurity by preparative LC

The solvent system used for Prep-LC is described in TABLE 2. Approximately 100g sample was loaded onto the Prep-LC and the fractions were collected. Purity of all fractions was determined using analytical LC. Solvent was evaporated under high vacuum Buchi Rota vapor -V-580.The remaining aqueous layer comprising of ammonium acetate salt was subjected to liquid-liquid extraction using methylene di-chloride. The organic layer was concentrated under high vacuum to dryness. The solid thus obtained was re-anlaysed on analytical LC. The chromatographic purity of this impurity is found to be 95% to 96%, which was relatively good enough for carrying out spectroscopic experiments.

Structural elucidation of impurity

The molecular ion of the impurity(M+1) at m/z 573.1 amu was 14 amu more than that of olmesartan medoxomil indicating the presence of methoxy group (see TABLE 3) in the impurity. The fragmentation pattern obtained by MS/MS data indicated a daughter ion at m/z 541.3 supporting the presence of one methoxy group. In addition to this, the characteristic OH stretching absorption band at 3290cm^{-1} was absent in the FT-IR spectrum of the impurity and it was present in that of olmesartan medoxomil (see TABLE 3). Presence of an -OMe functionality in the impurity was further confirmed by ^{1}H NMR. A singlet signal at δ2.91ppm in the impurity corresponding to 3 protons confirmed the presence of -OMe functionality (see figure 3). This peak was not observed in the olmesartan medoxomil ^{1}H NMR spectrum (see figure 4). Further to that absence of signal due to -OH group in the impurity in comparison to the olmesartan medoxomil confirmed the conversion of the -OH group to that of -OMe. This was further confirmed by ^{13}C NMR. Signal at δ47.73 ppm in the impurity confirmed the presence of -OMe group and there was no signal in the olmesartan medoxomil at this δ value. Based on the above spectral data the molecular formula of impurity was confirmed as $C_{30}H_{32}N_6O_6$ and the corresponding structure was characterized as 5-methyl-2-oxo-[1,3]dioxo-4-yl methyl-4-(1-methoxy -1-methyl-ethyl)-2-propyl-1-{4-[2'(1H-tetrazol-5-

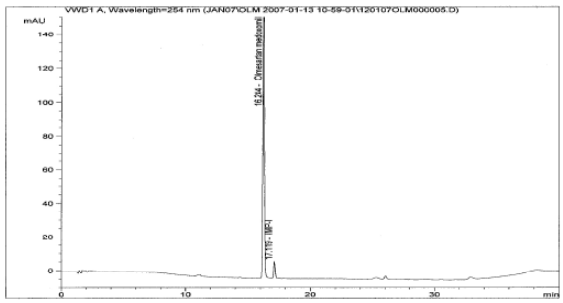

Figure 2: HPLC chromatogram of olmesartan medoxomil having 0.1% IMP-I

TABLE 3 : FT-IR and mass spectral data of olmesartan medoxomil, IMP-I

Compound	IR(per cm)	MS data (ESI)
Olmesartan medoxomil	2970(aliphatic C-H stretching) 3290(O-H stretching) 1832(C=O stretching) 1708(C=O stretching) 1476(C-H bending) 1393(C-O stretching) 1301(C-N stretching)	m/z(M+1)559.4,541.3, 429.2,275.0 207.1,195.3
IMP-I	2966(aliphatic C-H stretching) 1822(C=O stretching) 1711(C=O stretching) 1465(C-H bending) 1390(C-O stretching) 1306(C-N stretching)	m/z(M+1)572.9,541.0, 429.1,275.0,207.1,195.3

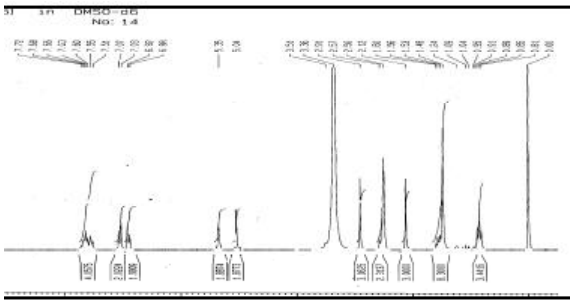

Figure 3 : ^{1}HNMR spectrum of IMP-I

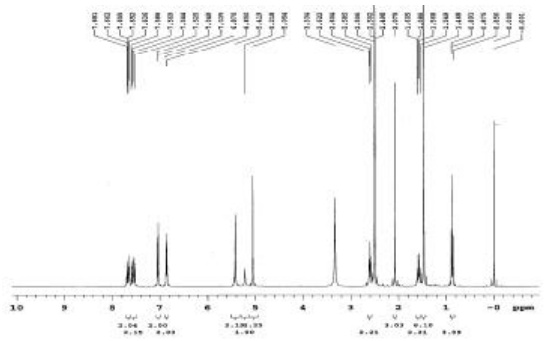

Figure 4 : ^{1}HNMR spectrum of olmesartan medoxomil

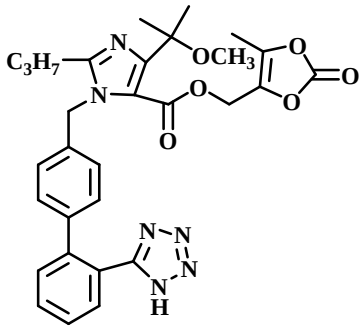

Figure 5 : Chemical structure of IMP-I

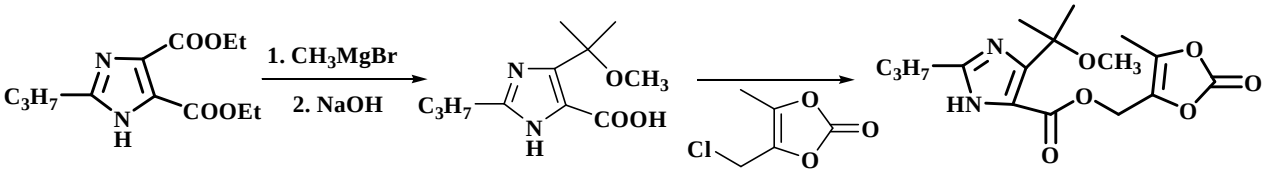

Figure 5: Chemical pathway for formation of IMP-I

TABLE 4 : Comparative ^{1}H NMR and ^{13}C NMR assignments for olmesartan medoxomil, IMP-I

Olmesartan medoxomil			IMP-I		
^{1}H	ppm	^{13}C PPM	^{1}H	ppm	^{13}C PPM
3H	0.87(t)	9.4	3H	0.86(t)	9.27
6H	1.47(s)	30.33	6H	1.53(s)	28.63
2H	1.59(m)	21.27	2H	1.60(m)	21.04
3H	2.08(s)	14.28	3H	2.12(s)	14.09
2H	2.60(t)	28.92	2H	2.57(t)	26.9
2H	5.05(s)	54.81	3H(OCH3)	2.91(s)	47.73
H(OH)	5.22(s)	-	2H	5.04(s)	54.31
2H	5.42(s)	48.7	2H	5.35(s)	50.35
2H	6.56(d)	129.64	2H	6.90(d)	129.53
2H	7.04(d)	126.11	2H	7.05(d)	126.3
2H	7.56(m)	128.45,131.12	2H	7.60(m)	128.32,131.06
2H	7.66(m)	131.22,131.70	2H	7.68(m)	131.56,133.85

T-triplet, s-singlet, m-multiplet and d-doublet

yl)phenyl] phenyl}methyl imidazole-5-carboxylate (figure 5).

Formation of impurity

The chemical path way for the formation of IMP-I is shown in the figure 6. The methoxy impurity was formed in the very first step where the Grignard reaction was performed on ethyl ester of imidazole(one of the key intermediate for making olmesartan medoximil bulk drug) for making gem dimethyl alcohol.

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