Synthesis and characterization of Tenofovir disoproxil fumarate impurities, anti HIV drug substance

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ABSTRACT

In the process development of Tenofovir disoproxil fumarate specified known, specified unknown and unidentified impurities found. The present work describes synthesis and characterizations of USP related known and unknown impurities; mono-POC Tenofovir (5), mono-POC methyl Tenofovir (6a), mono-POC ethyl Tenofovir, (6b), mono-POC isopropyl Tenofovir (6c), Tenofovir isopropyl isoproxil (7) and Tenofovir disoproxil carbamate (9). Identification of these impurities can be useful for quality control purpose in the manufacture of Tenofovir disoproxil fumarate.

Keywords: Antiretroviral Tenofovir disoproxil fumarate, impurity synthesis, characterization.

INTRODUCTION

Tenofovir disoproxil fumarate is a prodrug of Tenofovir and bis-isopropyloxycarbonylmethyl (bis-POC) moiety in improving the oral bioavailability of phosphonate nucleotides [1, 3]. Tenofovir disoproxil fumarate is USFDA approved potential antiretroviral agent. There is an ever increasing interest in impurities present in API. Recently, not only purity profile but also impurity profile become insist various regulatory requirements [4]. Majorly organic impurities are structurally similar with its API and comes from synthetic pathways or formulation. Due to structural similarity binds to receptor and decreases efficacy of drug and increases side effects, in order to decrease percentage of impurities it is important to identify the structures and origin of impurities [5]. According to ICH guide line, impurities in new drug products identification and quantification is mandatory, impurities above 0.1% considered as potentially toxic.

Tenofovir disoproxil fumarate, block reverse transcriptase, a crucial viral enzyme in human immunodeficiency virus 1 (HIV-1) and hepatitis B virus infections. Tenofovir is a highly potent antiviral agent, particularly for the therapy or prophylaxis of retroviral infections and belongs to a class of drugs called Nucleotide Reverse Transcriptase Inhibitors (NRTI) which blocks reverse transcriptase an enzyme crucial to viral production in HIV-infected people [6, 7]. Tenofovir is approved for commercial use as in the form of Tenofovir disoproxil fumarate (1), chemically known as 9-\{\{R\}-2-\{bis\{[(isopropoxy carbonyl)oxy]methoxy|phosphinyl|methoxy|propyl\}adenine fumarate with 9-R-(2-hydroxypropyl) adenosine as basic moiety. Tenofovir disoproxil fumarate is on the WHO’s list of essential medicines, the most important medications needed in a basic health system [7, 8].

MATERIALS AND METHODS

General. The reagents and solvents were purchased from sigma aldrich and dry triethyl amine and dry pyridine used for reactions. All reaction were carried out under nitrogen atmosphere. 1H NMR spectra were recorded on a Bruker Advance III 400 MHz with TMS as an internal standard. The IR spectra were recorded in the solid state as KBr dispersion using FT-IR IRAffinity-1, Shimadzu. The mass spectra were recorded on LC-MS API-2000, ABSciex.

O-[(Isopropoxycarbonyloxy)methyl]-O-methyl-[[[(R)-1-(6-Amino-9H-purin-9-yl)propan-2-yl]oxy]methyl]phosphonate (6a). Nature: Yellow liquid. IR (cm⁻¹): 3327, 2984, 1771, 1711, 1577, 1599, 1415, 1357, 1257, 1001, 898, 789, 648. ^1H NMR (CDCl₃) δ 8.27 (s, 1H, H-2), 7.90-796 (d, J = 5.2 Hz, 1H, H-8), 6.10 (brs, 2H, NH₂), 5.50-5.59 (m, 2H, OCH₃O), 4.80-4.91 (m, 1H, CH(CH₂)₂), 4.26-4.38 (tt, J = 3.2 & 2.0 Hz, 1H, CH₂N), 4.00-4.12 (m, 1H, CH₂N), 3.80-3.89 (m, 2H, OCH₂P), 3.88-3.86 (d, J = 8.8 Hz, 3H, OCH₃), 3.85-3.82 (m, 1H), 1.21-1.26 (m, 6H, CH₂(CH₂)₂), 1.19-1.67 (m, 3H). [M+H]^+ 417.9 [M+Na]^− 416.

O-[(Isopropoxycarbonyloxy)methyl]-O-ethyl-[[[(R)-1-(6-Amino-9H-purin-9-yl)propan-2-yl]oxy]methyl]phosphonate (6b). Nature: Yellow liquid. HPLC Purity: 87.07%. IR (cm⁻¹): 3327, 2982, 2936, 1757, 1577, 1599, 1470, 1246, 1097, 1037, 999, 947, 891, 829, 789, 719, 648. ^1H NMR (DMSO-d₆) δ 8.31 (s, 1H, H-2), 8.04 (s, 1H, H-8), 7.20 (s, 2H, NH₂), 5.55-5.46 (m, 2H, OCH₂O), 4.86-4.78 (m, 1H, CH(CH₂)₂), 4.31-4.23 (dd, J = 14.4 & 3.2 Hz, 1H, CH₂N), 4.21-4.11 (m, 1H, CH(CH₂)₂), 4.07-3.83 (m, 4H + 1H, 2 x OCH₂ + CH₂N), 1.25-1.23 (dd, J = 6.4 & 2.8 Hz, 6H, 2 x CH₃), 1.19-1.12 (m, 3H, CH₃CH₂), 1.09-1.07 (dd, J = 6.4 & 1.6 Hz, 3H, CH₂CH₃). Mass: [M+H]^+ 432.

O-[(Isopropoxycarbonyloxy)methyl]-O-ethyl-[[[(R)-1-(6-Amino-9H-purin-9-yl)propan-2-yl]oxy]methyl]phosphonate (6c). Nature: Pale yellow liquid. HPLC Purity: 83.15%. IR (cm⁻¹): 3329, 3166, 2981, 2935, 1757, 1643, 1599, 1417, 1247, 1143, 1099, 1028, 983, 902, 719. ^1H NMR (CDCl₃) δ 8.33 (s, 1H, H-2), 8.00 (s, 1H, H-8), 6.03 (brs, 2H, NH₂), 5.71-5.58 (d, 2H, dd, J = 17.2 Hz, OCH₂O), 4.98-4.87 (m, 1H, CH(CH₂)₂), 4.83-4.68 (m, 1H, CH(CH₂)₂), 4.30-4.48 (dd, 1H, J = 15.2 & 4.4 Hz, CH₂N), 4.00-4.28 (m, 2H, OCH₂P), 3.82-3.98 (m, 1H, CH₂N), 3.51-3.66 (m, 1H, CH(CH₃)), 1.13-1.42 (m, 15H, CH₂x(CH₂)₂ + CH₂), [M+H]^+ 446.0 [M+Na]^+ 468, [M+K]^+ 484.

5-[[1R]-2-(6-Amino-9H-purin-9-yl)-1-methylhexyloxymethyl]-2,4,6,8-tetraoxa-5-phosphonanediethylpropyl Ester 5-Oxide (7). Triethylamine (3.53 ml, 0.0254 moles) was added to mono-POCPMPA (5) (3.6 g, 0.0089 moles) solution of N-methyl pyrrolidone (10.8 ml, 30 volumes) and stir the reaction mixture for 30 minutes at room temperature. Reaction mixture was then heated to 54 °C, chloromethyl n-propyl carbonate (1.54 ml, 0.0116 moles) was added at same temperature and maintained 5.0 hours. Monitor the reaction progress by TLC (ethyl acetate/methanol 40:60 v/v). Ethyl acetate (12.0 ml) was added in reaction mixture at then cooled to 10-15 °C and...
stirred for 1 h. Filter the precipitated white solid and washed with ethyl acetate. Filtrate was concentrated and the crude product purified by flash column chromatography using methanol-ethyl acetate as gradient mobile phase to obtain pure compound, yield 11%.

Nature: Pale yellow liquid. HPLC Purity 91.90%. IR (cm⁻¹): 2972, 2937, 1759, 1614, 1662, 1421, 1246, 1153, 1099, 1028, 952, 893, 788, 646. ¹H NMR (CDCl₃) 6: 8.29 (s, 1H, H-2), 7.80 (s, 1H, H-8), 6.45 (bs, 2H, NH₂), 5.80-5.58 (d, J = 16.8 Hz, 4H, OCH₂), 4.98-4.80 (m, 1H, CH(CH₂)₂), 4.48-4.30 (d, 1H, J = 19.2 Hz, CH₂N, 4.21-4.15 (m, 2H, OCH₂P), 3.98-3.82 (m, 2H, OCH₂), 3.75-3.65 (dd, 1H, J = 14 & 4.4 Hz, CH₂N), 3.47-3.38 (m, 1H, CHCH₂), 1.79-1.67 (m, 2H, CH₂), 1.33-1.27 (dd, 6H, J = 8.0 Hz, CH(CH₂)₂), 1.24-1.20 (d, 3H, J = 7.2 Hz, CH₃), 1.12-0.87 (t, 3H, J = 6.4 Hz, CH₃). [M+Na]⁺ 520.0 [M+Na]⁺ 542.0, [M+K⁺]⁺ 558.0

O, O-Bis (isopropoxyoxycarbonyloxymethyl)((R)-1-[6-isopropoxyoxycarbonyl amino]-9H-purin-9yl) propan-2-yl oxy]methylphosphonate (9)
Tenofovir disoproxil fumarate (1) (1.0 g, 0.0019 moles) suspended in N-methyl pyrrolidone (3 ml, 3.0 volumes) at room temperature then triethylamine (0.9 ml, 0.0060 moles) was added dropwise to give clear solution of reaction mass. Isopropyl chloroformate was added dropwise (0.9 ml, 0.0029 moles) and stir the reaction mass at 60 °C for 6 h. Monitor the reaction progress by TLC (dichloromethane-methanol 95:5 v/v). Reaction mixture was diluted in water 20 ml and extracted in ethyl acetate (3 x 25 ml), dried over sodium sulphate and concentrated to obtain sticky compound. The crude product was purified by column chromatography using gradient dichloromethane and ethylacetate to obtain pure product 0.43 g, yield 37%.

Nature: Pale yellow liquid. HPLC Purity 82.16%. IR (cm⁻¹): 2978, 2853, 1755, 1613, 1516, 1458, 1358, 1227, 1176, 1028, 954, 897, 789, 748. ¹H NMR (CDCl₃) 6: 8.75 (s, 1H, H-2), 7.81 (s, 1H, H-8), 5.69-5.56 (m, 4H, 2xCH₂), 5.17-5.07 (m, 1H, CH), 4.96-4.86 (m, 2H, CH₂), 4.46-4.40 (dd, 1H, J = 14.4 & 2.8 Hz, CH), 4.22-4.13 (dd, 2H, J = 14.8 & 7.6 Hz, CH), 4.00-3.91 (m, 2H, CH₂), 3.73-3.65 (dd, 1H, J = 13.6 & 9.2 Hz, CH), 1.44-1.36 (d, 3H, CH₃), 1.33-1.20 (m, 18H, 4 x CH₃) [M+Na]⁺ 628.8.

RESULTS AND DISCUSSION

Three major steps are involved in Tenofovir disoproxil fumarate (1) synthesis [9, 10] (Scheme 1) are reacting 9-[2-(R)-(hydroxy)propyl] adenine (2) with diethyl-p-toluen sulfonyl-oxymethyl phosphonate, dealkylating the resulting compound of formula (3) with acid to obtain Tenofovir (4) which on reacting with chloromethyl isopropyl carbonate followed by treating with fumaric acid gives Tenofovir disoproxil fumarate (1)

9-yl) propan-2-yloxy]methylphosphonate and Impurity 6c Tenofovir isopropyl isoproxil or mono-POC isopropyl impurity. O-(Isopropoxycarbonyloxymethyl)-O-isopropyl-[(R)-1-(6-amino-9H-purin-9-yl) propan-2-yloxy]methyl phosphonate are monoether impurities derived from process related impurities. Impurities 6a, 6b and 6c synthesized from intermediate 5. Impurity 7 n-POC-POC Tenofovir chemically known as 5-[[1(R)-2-[(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]-2,4,6,8-Tetraoxa-5-phosphooxanenedioic acid 1-(1-methylethyl) 9-propyl ester 5-oxide synthesized from intermediate 5 and chloromethyl n-propyl carbonate [12]. Impurity 8 Tenofovir disoproxil carbamate, chemically known as O, O-Bis(isopropoxycarbonyloxymethyl)[(R)-1-[(6-isopropoxycarbonyl amino)-9H-purin-9-yl] propan-2-yloxy]methylphosphonate and are process related impurities synthesized treating Tenofovir disoproxil API with isopropyl chloroformate in basic condition. All impurities are as listed in Scheme 2.
Scheme 4. Synthesis of n-POC-POC Tenofovir (7)

Scheme 5. Tenofovir disoproxil carbamate (9)

CONCLUSION

All of the above synthesized impurities Tenofovir isoproxil monoester, mono-POC methyl (6a), mono-POC ethyl (6b) and mono-POC i-propyl ether (6c), n-POC-POC Tenofovir (7) and Tenofovir disoproxil carbamate (9) can be used for impurity profiling of Tenofovir disoproxil fumarate.

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REFERENCES