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SYNTHESIS AND CHARACTERIZATION OF PROCESS-RELATED IMPURITIES IN AZELNIDIPINE

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ABSTRACT

Azelnidipine is a new dihydropyridine derivative with calcium antagonistic activity. It is chemically designated as 3-[1-(diphenyl methyl)azetidin-3-yl] 5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)1,4-dihydropyridine-3,5-dicarboxylate.It is a dihydropyridine calcium channel antagonist with selectivity for L-typecalcium channels. The recommended dosing of Azelnidipine is 16 mg per day. As per International Conference on Harmonization (ICH) guidelines for impurities in new drug substances, reporting threshold is 0.05% and identification threshold is 0.10% for maximum daily dose≤2 g/day. This paper describes the synthesis and structure characterization of Azelnidipine, stage-I intermediate (propan-2-yl (2Z)-2-(3-nitrobenzylidene)-3-oxobutanoate), impurities (Impurities-1 to 9) were using FT-IR, Mass and NMR spectral data.

***KEY WORDS**: Azelnidipine, Impurities, synthesis, Characterization.*

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1.0 INTRODUCTION

Azelnidipine is a new dihydropyridine derivative with L-type calcium antagonistic activity [1 to 4]. It is chemically designated as 3-[1-(diphenyl methyl)azetidin-3-yl] 5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)1,4-dihydropyridine-3,5-dicarboxylate. Its molecular formula is $C_{33}H_{34}N_4O_6$ and molecular weight is 582.65 amu. Azelnidipine is offered under the registered trademark CALBLOCK(R) by Sankyo Co. Ltd. of Japan. CALBLOCK(R) is offered as an oral tablet administered once daily for the treatment of hypertension and related diseases. The recommended dosing of Azelnidipine is 16 mg per day. The presence of impurities in an Active Pharmaceutical Ingredient (API) will influence the quality and safety of the drug product. As per International Conference on Harmonization (ICH) guidelines for impurities in new drug substances, reporting threshold is

0.05% and identification threshold is 0.10% for maximum daily dose≤2 g/day [5]. Pure Impurities are required to check the analytical performance characteristics such as specificity, linearity, range, accuracy, precision, limit of detection, limit of quantification, robustness, system suitability testing, and relative retention factor [6]. Impurities (impurity-1, impurity-2 and impurity-7) were isolated from crude samples/enriched mother liquors by using column chromatography; Impurities (impurity-3, impurity-4, impurity-5, impurity-6 and impurity-9) were prepared by synthetic procedures. This paper describes the synthesis and structure characterization of Azelnidipine raw material, raw material impurity (impurity-1), intermediate (stage-I), Stage-I impurities (Impurity-2 to 6), Azelnidipine (stage-II) impurities (Impurity-7 to 9) structures by using FT-IR, Mass and NMR spectral data.

2.0 MATERIALS AND METHODS

2.1. Sample, chemicals and reagents

Azelnidipine was synthesized in Chemical Research Department of Mylan laboratories limited, Plot No.34-A, ANRICH Industrial Estate, Jinnaram (Mandal), Bollaram, Medak District – 502325, Telangana, India. Deuterated dimethyl sulfoxide (DMSO-d_6) solvent was from Cambridge Isotope Laboratories, Inc. (USA). The scheme for synthesis of Azelnidipine [7 to 12] is shown in Fig. 1 and synthetic procedures for Impurities (impurity-3, impurity-4, impurity-5, impurity-6 and impurity-9) are in Fig. 2.

2.2. NMR spectroscopy: The NMR experiments were performed on a Bruker AVANCE-300 instrument with a 5-mm BBO probe head equipped with shielded *Z*-gradient coil at 298 K using Deuterated dimethyl sulfoxide (DMSO-d_6) as solvent and tetra methyl silane (TMS) as internal standard. The data were collected by XWIN-NMR software (Bruker) and processed with Topspin running on a PC with Microsoft Windows^{xp}. The ^{1}HNMR analysis, 16 transients were acquired with a 1-s-relaxation delay using 32 K data points. The 90° pulse duration was of 11 μs and spectral width 6.000 kHz. The ^{13}C NMR and DEPT experiments were carried out with a spectral width of 16.500 kHz using 64 K data points. The two-dimensional experiments were performed using Bruker standard pulse sequences and parameters. The ^{1}H–^{1}H bond correlations confirmed by gCOSY experiment. The protonated carbon positions were confirmed by gHSQC experiment. The non-protonated carbons were confirmed by gHMBC experiment. The ^{1}H chemical shifts are reported in ppm with reference to tetra methyl silane (δ0.0 ppm). The ^{13}C chemical shifts were referenced to the central peak of the solvent molecule of DMSO-d_6 at (δ39.50ppm).

2.3. Mass spectrometry: Mass spectra were recorded on Agilent 1100 Series LC-MSD-TRAP-SL system mass spectrometer equipped with a Turbo ion spray interface at 375°C. Detection of ions was performed in electro spray ionization, positive ion mode and in negative ion mode.

2.4. Gas chromatography with Mass spectrometry: Mass spectra were recorded on Perkin-Elmer GC-MS System in chemical Ionization mode.

2.5. IR spectroscopy: The FT-IR spectra were recorded on Perkin Elmer Spectrum One FT-IR Spectrometer by using Potassium bromide pellet method.

3.0 RESULTS AND DISCUSSION

3.1 Structure Elucidation

3.1.1 Methyl Aceto Acetate: This is one of the key raw material in the synthesis of Azelnidipine (Fig.1). The chemical Ionization mode mass displayed 116$[M]^+$indicating the molecular weight of the compound as 116. The ^{1}H NMR data of this raw material is having eight protons in aliphatic region. A singlet at δ2.17ppm (H-1) with integration of three protons, H-2 at δ2.67ppm as a singlet with integration of two protons, H-5 at δ3.63ppm as a singlet with integration of three protons. The ^{13}C NMR spectrum displays the two methyl groups C-1 at δ25.96ppm, C-5 at δ 51.71ppm, one methylene group C-3 at δ49.31ppm and two carbonyl groups C-2 at δ201.45ppm, C-4 at δ167.67ppm. The above spectral data confirms the Methyl Aceto Acetate as methyl 3-oxobutanoate with molecular formula $C_5H_8O_3$ and molecular weight 116.

3.1.2 Impurity-1 (Raw material Impurity): This impurity is a raw material (methyl acetoacetate) impurity. The chemical Ionization mode mass displayed 168$[M]^+$indicating the molecular weight of the compound as 168, which is 52 amu units higher than Methyl Aceto Acetate. The ^{1}H NMR data of this impurity is having six aliphatic protons as singlet's at H-1 δ2.28ppm, H-8 δ2.67ppm, one aromatic proton at H-3 δ5.97ppm and one exchangeable proton at δ16.70ppm. By comparing the ^{13}C NMR of this impurity with methyl Aceto acetate, one methylene carbon was disappeared; one extra aromatic carbon C-3 at δ101.36 ppm, one methyl carbon C-8 at δ29.98ppm and two carbonyls at δ161.13 ppm and δ205.17 ppm were appeared; C-5 carbon signal deshielded from δ51.71ppm to δ99.78ppm. Based on the above observations from spectral data confirms the Impurity-1 as 3-acetyl-4-hydroxy-6-methyl-2*H*-pyran-2-one with molecular formula C_8H_8O4 and molecular weight 168.

3.1.3 Stage-1: This is an intermediate in the synthesis of Azelnidipine (Fig.1). The ESI mass spectrum in positive mode displayed an adduct ions at m/z 300$[M+Na]^+$, m/z 316$(M+K)^+$indicating the molecular weight of the compound as 227. The fragmentation pathway of the protonated molecular ion at m/z 228 was obtained: m/z at 201.The ^{1}H NMR data is having ten aliphatic protons related to isopropyl (H-12, H-13, H-13') and methyl group (H-10). The aromatic region has five protons. The chemical shift values of H-1, H-3 are deshielded in ^{1}H NMR spectrum due to the presence of $–NO_2$ group on C-2 carbon atom. The ^{13}C NMR spectrum of this stage-I displays the three methyl carbons, C-10 at δ26.10ppm, C-13,13' at δ21.18ppm; one methane carbon δ69.33ppm; one alkene carbon C-7 at δ138.68ppm; four aromatic carbons C-1 at δ124.99ppm, C-3 at δ123.22ppm, C-4 at δ130.66ppm, C-5 at δ135.50ppm; six quaternary carbons including two carbonyl carbons C-2 at δ147.96ppm, C-6 at δ136.64ppm, C-8 at δ134.56ppm, C-9 at δ195.82ppm and C-8 at δ165.95ppm which were confirmed by DEPT-135 experiment. The above spectral data confirms the stage-I as propan-2-yl (2*Z*)-2-(3-nitrobenzylidene)-3-oxobutanoate with molecular formula $C_{14}H_{15}NO_5$ and molecular weight 277.

3.1.4 Azelnidipine: The ESI mass spectrum in positive mode displayed a protonated molecular ion at m/z 583$[M+H]^+$ in negative mode displayed a deprotonated molecular ion at m/z 581$[M-H]^-$ and 695$[M+CF_3COOH-H]^-$ indicating the molecular weight of the compound as 582. The fragmentation pathway of the protonated molecular ion at m/z 583 was obtained: m/z at 167. The structure of Azelnidipine was confirmed by using 1H

NMR, ^{13}C NMR, DEPT and Mass spectral data and this data also matching with Literature [12] reported data. Solid state NMR spectral data has been discussed in literature [13].

3.1.5 Impurity-2: ESI mass spectrum of Impurity-2 in positive ion mode showed a protonated molecular ion at m/z 422 $[M+H]^+$ indicating the molecular weight of the compound as 421. The fragmentation pathway of the protonated molecular ion at m/z 422 was obtained: m/z at 403,362.The molecular weight of this impurity was 144 amu more than that of stage-I. The FT-IR spectral data showed a sharp OH stretching at 3489 cm^{-1}. By comparing the ^1H NMR of this impurity with stage-I; one extra isopropyl group protons H-19 at δ4.57-4.65ppm, H-20,20' as a doublets at δ0.73ppm,δ0.88ppm; one extra methylene protons H-15 at 2.37-2.94ppm; one methine proton H-7 as a doublet and shielded from δ7.92-7.97ppm to δ3.97ppm; two extra methane protons H-8 as a doublet at δ3.43ppm, H-17 as a doublet at δ4.07ppm were appeared. In HCOSY spectrum H-8 at δ3.43ppm shows the correlation with H-7 at δ2.20ppm; H-7 at δ3.97ppm shows the correlation with H-17 at 4.05-4.07ppm, indicating that H-7, H-8 and H-17 are in one spin system. In ^{13}C NMR data showed one extra aliphatic quaternary carbon C-9 at δ72.62ppm, one aliphatic methylene carbon C-15 at δ54.22ppm and one extra isopropyl group carbons signals C-19 at δ67.11ppm, C-20,20' at δ21.04ppm, δ21.18ppm; one extra carbonyl carbon C-18 at δ167.42ppm; three methine carbon signals at C-7 at δ43.90ppm, C-8 at δ55.610ppm and C-17 at δ61.36ppm were observed. In HMBC spectrum H-15a at δ2.35-2.39ppm, H-15b at δ2.92-2.96ppm, H-17 at δ4.05-4.09ppm shows the correlation with C-16 at δ202.76ppm and H-15a at δ2.35-2.39ppm, H-15b at δ2.92-2.96ppm also shows the correlation with C-9 at δ72.62ppm. As seen the from HCOSY and HMBC correlations cyclisation occurred and six member ring was formed with C7,C8,C9,C15,C16 and C-17.The above spectral data confirms the Impurity-2 as Dipropn-2-yl-4-hydroxy-4-methyl-2-(3-nitrophenyl)-6-oxocyclohexane-1,3-dicarboxylate with molecular formula $C_{21}H_{27}NO_8$ and molecular weight 421.The probable mechanisms for the formation of the impurity were shown in Figure.2.

3.1.6 Impurity-3: ESI mass spectrum of Impurity-3 in positive ion mode showed a protonated molecular ion at m/z 264 $[M+H]^+$ indicating the molecular weight of the compound as 263. The fragmentation pathway of the protonated molecular ion at m/z 263 was obtained: m/z at 235 and 218. The molecular weight of this impurity was 14 amu less than that of stage-I. The ^1H NMR spectrum of this impurity showed a triplet H-13 at δ1.20ppm with integrating for three protons and a quartet H-12 atδ4.24-4.31ppm with integration of two protons. By comparing the ^{13}C NMR spectrum of this impurity with stage-I, one extra methylene carbon signal C-12 at δ61.51ppm was appeared. The above spectral data confirms the Impurity-3 as ethyl-2-(3-nitrobenzylidene)-3-oxobutanoate with molecular formula C13H13NO5 and molecular weight 263.

3.1.7 Impurity-4: ESI mass spectrum of Impurity-4 in positive ion mode showed a adduct ion at m/z 272 $[M+Na]^+$ indicating the molecular weight of the compound as 249. The molecular weight of this impurity was 28 amu less than that of stage-I. By comparing the ^1H NMR spectrum of this impurity with stage-I, Isopropyl moiety protons were disappeared; one singlet at δ3.79ppm with integrating of three protons were appeared. In this impurity ^{13}C NMR spectrum, Isopropyl moiety carbon signals were disappeared and one methyl carbon signal C-12 at δ52.58ppm was appeared. As seen from the above spectral information Impurity-4 confirmed as methyl -2-(3-nitrobenzylidene)-3-oxobutanoate with molecular formula $C_{12}H_{11}NO_5$ and molecular weight 249.

3.1.8 Impurity-5: ESI mass spectrum of Impurity-5 in positive ion mode showed a protonated molecular ion at m/z 278 $[M+H]^+$ indicating the molecular weight of the compound as 277. The fragmentation pathway of the protonated molecular ion at m/z 278 was obtained: m/z at 235, 149. The molecular weight of this impurity is equal to the molecular weight of stage-I. By comparing the ^1H NMR spectrum of this impurity with stage-I, change in aromatic protons multiplicity pattern indicates that the attachment of $–NO_2$ group was changed instead of meta position. In ^{13}C NMR spectrum of this impurity C-1 carbon signal appeared as a quaternary carbon signal and deshielded from δ124.99ppm to δ146.77ppm; C-2 carbon signal appeared as an aromatic carbon signal and shielded from δ147.96ppm to δ124.85ppm indicating that $–NO_2$ group attached on the ortho position on aromatic ring instead of meta position. The above spectral data confirms the Impurity-5 as propan-2-yl -2-(2-nitrobenzylidene)-3-oxobutanoate with molecular formula $C_{14}H_{15}NO_5$ and molecular weight 277.

3.1.9 Impurity-6: ESI mass spectrum of Impurity-6 in positive ion mode showed an adduct ions at m/z 300$[M+Na]^+$, 316$[M+K]^+$ indicating the molecular weight of the compound as 277. In^1H NMR spectrum of this Impurity –5, H-1,5 appeared as a doublet at δ8.26ppm with integration of two protons; H-2,4 appeared as a doublet at δ7.69ppm with integration of two protons; remaining all protons when compared to stage-I appeared at same chemical shift values with equal number of protons. In ^{13}C NMR spectrum of this impurity, C-1,5 carbon signal appeared at δ130.61ppm; C-2,4 carbon signal appeared atδ123.90ppm. This concludes that $–NO_2$ group attached in para position on aromatic ring instead of meta position. The above spectral data confirms the Impurity-6as propan-2-yl -2-(4-nitrobenzylidene)-3-oxobutanoate with molecular formula $C_{14}H_{15}NO_5$ and molecular weight 277.

3.1.10 Impurity-7: ESI mass spectrum of Impurity-7 in positive ion mode showed a protonated molecular ion at m/z 643$[M+H]^+$ and in negative mode deprotonated molecular ion at m/z 641$(M+H)^-$ indicating the molecular weight of the compound as 642. The fragmentation pathway of the protonated molecular ion at m/z 643 was obtained: m/z at 510. The molecular weight of this impurity has 60 amu higher than that of Azelnidipine. By comparing the ^1H NMR spectrum of this impurity with Azelnidipine one extra singlet at δ2.02ppm with integration of three protons, one exchangeable proton NH' at 7.31.7.36ppm were observed and H-14' deshielded to higher chemical shift value at δ4.21-4.23ppm and remaining all protons appeared at same chemical shift values with same number of protons. In ^{13}C NMR spectrum of this Impurity displays one extra methyl carbon at C-27 at δ20.58ppm, one carbonyl carbon at δ170.09ppm were appeared; C-14' chemical shift value deshielded from δ60.18ppm to δ63.41ppm; and remaining all carbon signals when compared with Azelnidipine appeared at same chemical shift values. As seen from the NMR and Mass spectral data, azetidine moiety ring was opened and acetyl group attached to one of the methylene carbon in azetidine moiety. The above spectral data confirms the Impurity-7 as 3-{1-(acetyloxy)-3-[(diphenylmethyl)amino]propan-2-yl}5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate with molecular formula $C_{35}H_{38}N_4O_8$ and molecular weight 642.

3.1.11 Impurity-8: This impurity is an Azelnidipine (Fig.1) impurity and this is formed during the synthesis of stage-II (Azelnidipine) and there is a chance for carryover to product. ESI mass spectrum of Impurity-8 in positive ion mode showed a protonated molecular ion at m/z 601$[M+H]^+$ indicating the molecular weight of the compound as 600. The molecular weight of this impurity has 19 amu higher than that of Azelnidipine. The

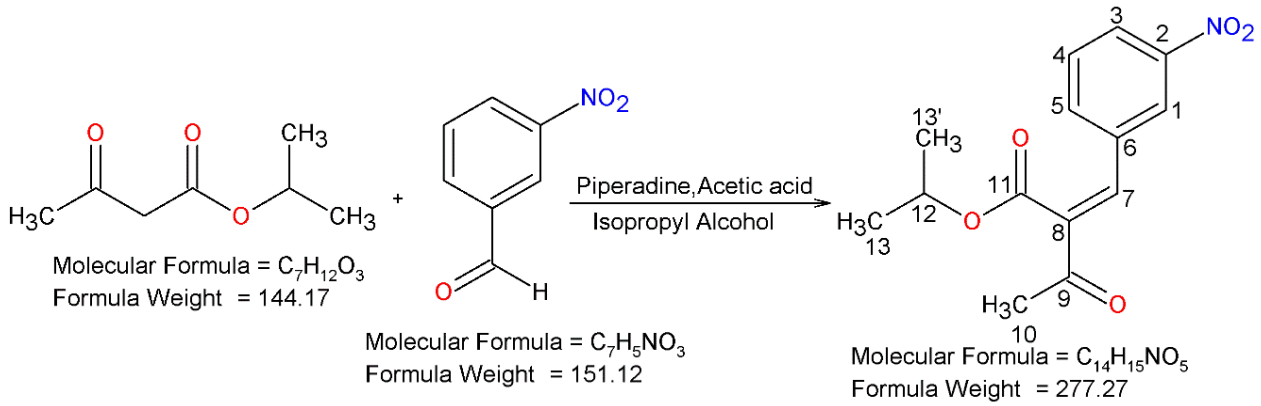
fragmentation pathway of the protonated molecular ion at m/z 601 was obtained: m/z at 239.By comparing the ^{1}H NMR spectrum of this impurity with Azelnidipine, two extra methane proton signals H-7 as a doublet at δ4.19ppm; H-8 at δ2.54ppm; one extra exchangeable proton as a singlet at δ5.59ppm which is confirmed by D_2O exchange experiment were appeared and the remaining all protons appeared at same chemical shift values with same number of protons. When compared to the ^{13}C NMR spectrum of Azelnidipine, in this impurity C-7 carbon signal slightly deshielded from δ38.82ppm to δ39.50ppm; C-8 carbon signal appeared as methane carbon instead of quaternary carbon signal and shielded from δ102.78ppm to δ58.71ppm; C-9 carbon signal shielded from δ145.31ppm to δ77.46ppm; remaining all carbon signals were appeared with same chemical shift values. As seen from the NMR and Mass spectral data, one hydroxyl group was attached on C-9 carbon atom. The above spectral data confirms the above impurity-8 as 5-[1-(diphenylmethyl)azetidin-3-yl] 3-propan-2-yl 6-amino-2-hydroxy-2-methyl-4-(3-nitrophenyl)1,2,3,4-tetrahydropyridine-3,5-dicarboxylate with molecular formula $C_{33}H_{36}N_4O_7$ and molecular weight 600.

3.1.12 Impurity-9: ESI mass spectrum of Impurity-11 in positive ion mode showed a protonated molecular ion at m/z 583 $[M+H]^+$, in negative ion mode showed a deprotonated molecular ion peak at m/z *581* $[M-H]^-$, indicating the molecular weight of the compound as 582.The fragmentation pathway of the protonated molecular ion at m/z 583 was obtained: m/z at 167. The molecular weight of this impurity is equal to the molecular weight of Azelnidipine. By comparing the ^{1}H NMR spectrum of this impurity with Azelnidipine, change in aromatic protons multiplicity pattern indicates that the attachment of $–NO_2$ group was changed instead of meta position. In ^{13}C NMR spectrum of this impurity C-1 carbon signal appeared as a quaternary carbon signal and deshielded from δ122.49ppm to δ147.00ppm; C-2 carbon signal appeared as an aromatic carbon signal and shielded from δ151.74ppm to δ123.84ppm indicating that $–NO_2$ group attached on the ortho position on aromatic ring instead of meta position and the remaining all carbons signals were appeared with same chemical shift values when compared to the Azelnidipine. The above spectral data confirms the above impurity-9 as 3-[1-(diphenylmethyl)azetadin-3-yl] 5-propan-2-yl 2-amino-6-methyl-4-(2-nitrophenyl)1,4-dihydropyridine-3,5-dicarboxylate with molecular formula $C_{33}H_{34}N_4O_6$ and molecular weight 582.

Azelnidipine raw material, raw material impurity (impurity-1), Stage-I impurity (impurity-2), Azelnidipine (stage-II) impurities (impurity-7, impurity-8) structures, IUPAC names with molecular formula and molecular weight were tabulated in Table.1; Table 2 contains the FT-IR data for Azelnidipine, stage-I, raw material and impurities (impurity-1 to 9); The ^{1}H, ^{13}C NMR chemical shifts in δ (ppm) of Azelnidipine Raw material and its impurity (impurity-1) were in Table.3; The ^{1}H NMR, ^{13}C chemical shifts in δ (ppm) of Azelnidipine stage-I and its impurities (impurity-2 to 6) in Table.4, 5; The ^{1}H NMR, ^{13}C NMR chemical shifts in δ (ppm) of Azelnidipine and its impurities (impurity-7 to 9) were tabulated in Table.6 and Table.7 respectively.

Fig. 1: The synthetic scheme of Azelnidipine**:**

Stage-I: Preparation of Isopropyl 2-(3-nitobenzylidine) acetoacetate



Stage-II: Preparation of 3-(1-benzhydryl-3-zetidinyl) 5-Isopopyl-2-amino-6-methyl-4-(3-nitophenyl)1,4-dihydropyridine-3,5-dicaboxylate

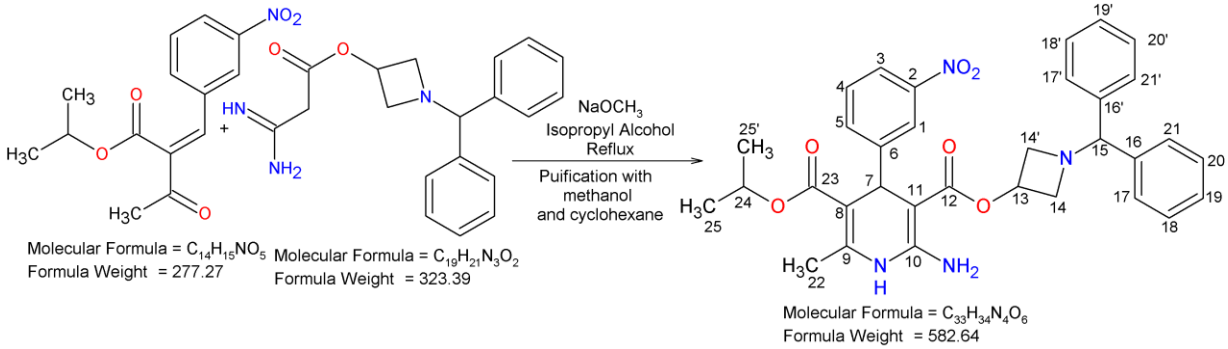
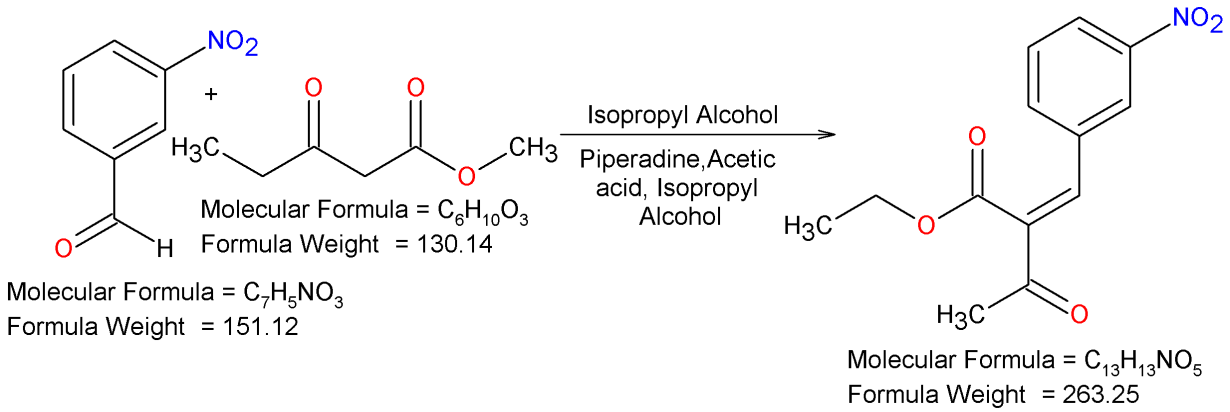
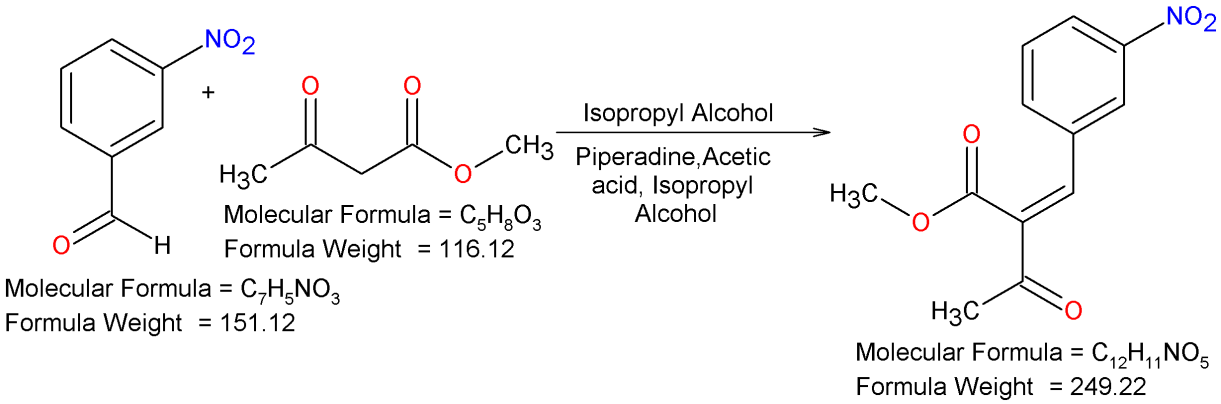


Fig.2: Synthetic procedures for impurities (3,4,5,6 and 8)

Impurity-3



Impurity-4

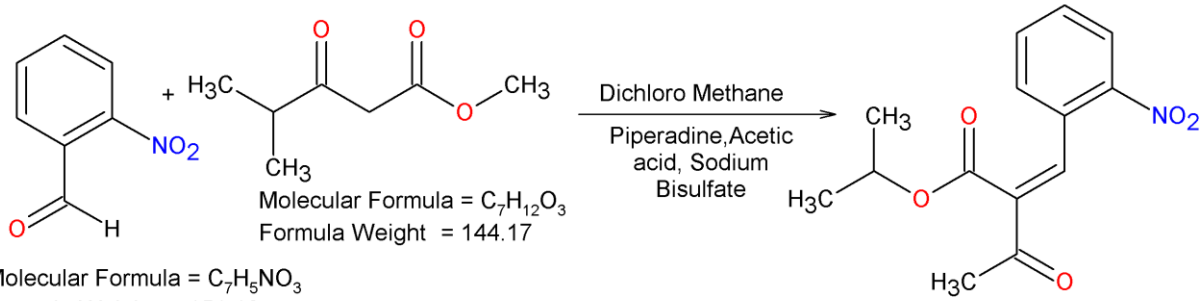


Molecular Formula = $C_5H_8O_3$
Formula Weight = 116.12

Molecular Formula = $C_7H_5NO_3$
Formula Weight = 151.12

Molecular Formula = $C_{12}H_{11}NO_5$
Formula Weight = 249.22

Impurity-5

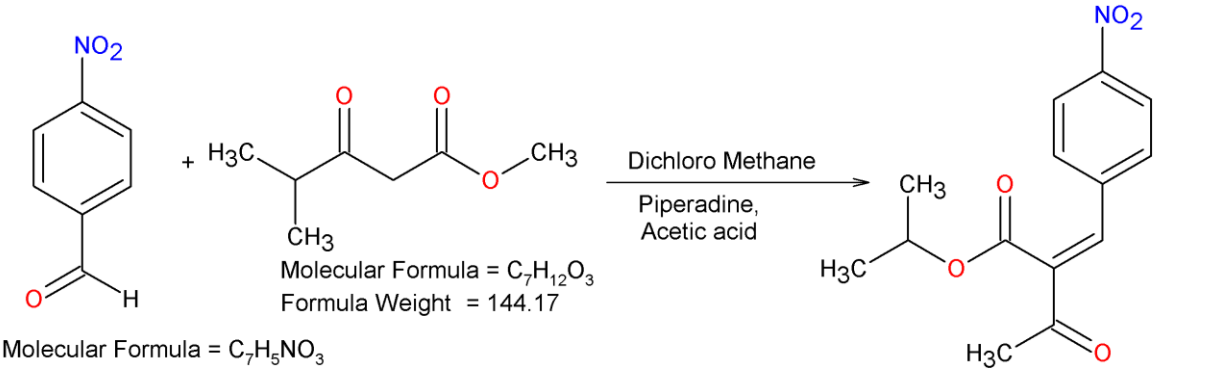


Molecular Formula = $C_7H_{12}O_3$
Formula Weight = 144.17

Molecular Formula = $C_7H_5NO_3$
Formula Weight = 151.12

Molecular Formula = $C_{14}H_{15}NO_5$
Formula Weight = 277.27

Impurity-6



Molecular Formula = $C_7H_{12}O_3$
Formula Weight = 144.17

Molecular Formula = $C_7H_5NO_3$
Formula Weight = 151.12

Molecular Formula = $C_{14}H_{15}NO_5$
Formula Weight = 277.27

Impurity-9

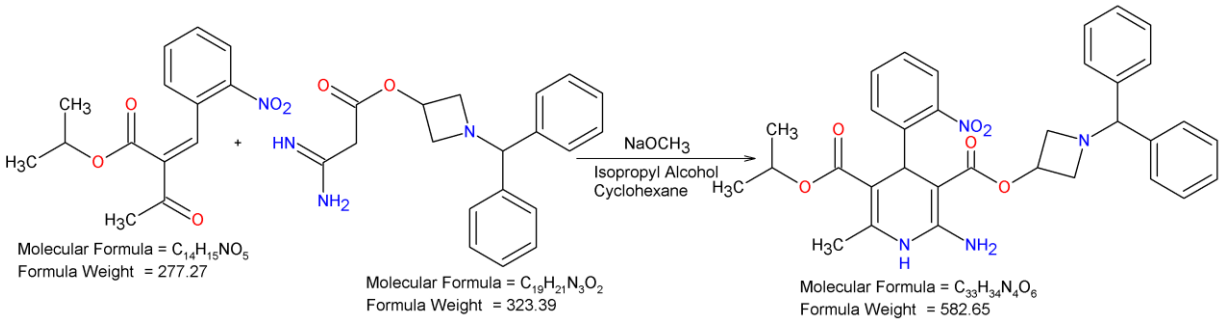


Fig.3: schematic diagram for the formation of the Impurity-2:

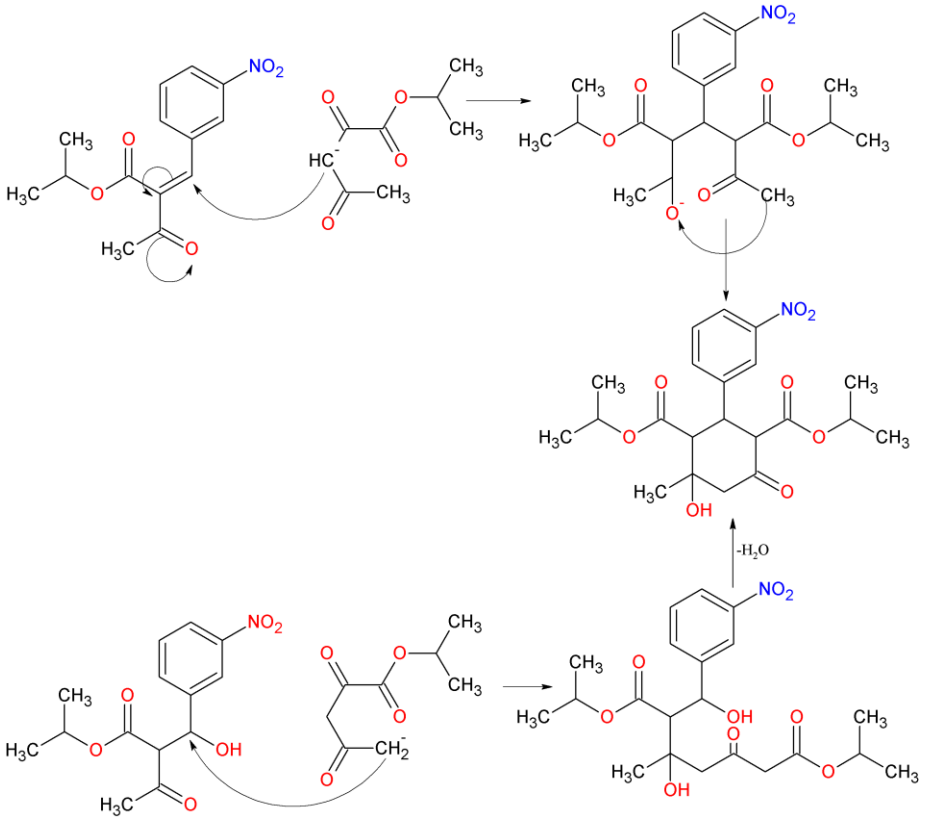
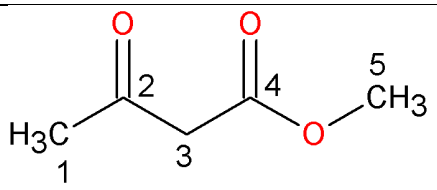
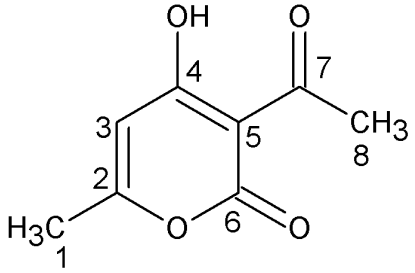
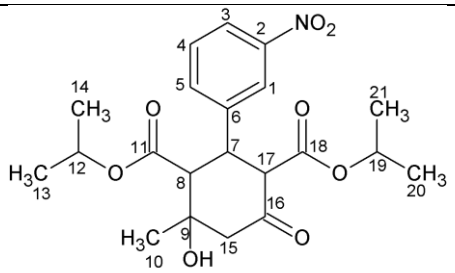


Table.1: Azelnidipine raw material, raw material impurity (impurity-1), intermediate (stage-I), Stage-I impurity (impurity-2), Azelnidipine (stage-II) impurities (Impurity-7, impurity-8) structures, IUPAC names with molecular formula and molecular weight.

S.No	Name of the compound	IUPAC Name	Structure
1.	Methyl Aceto Acetate	methyl 3-oxobutanoate	 Molecular Formula = $C_5H_8O_3$ Formula Weight = 116.12
2.	Impurity-1	3-acetyl-4-hydroxy-6-methyl-2*H*-pyran-2-one	 Molecular Formula = $C_8H_8O_4$ Formula Weight = 168.15
3.	Impurity-2	Dipropn-2-yl-4-hydroxy-4-methyl-2-(3-nitrophenyl)-6-oxocyclohexane-1,3-dicarboxylate	 Molecular Formula = $C_{21}H_{27}NO_8$ Formula Weight = 421.44

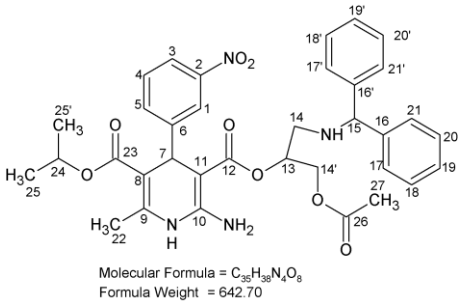
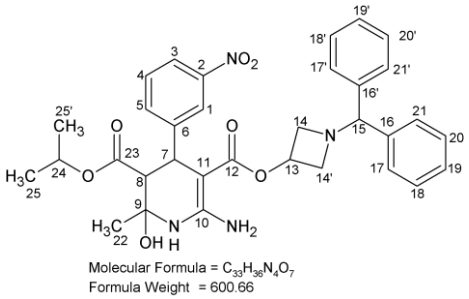
4.	Impurity-7	3-{1-(acetyloxy)-3-[(diphenylmethyl)amino]propan-2-yl}5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	
5.	Impurity-8	5-[1-(diphenylmethyl)azetidin-3-yl] 3-propan-2-yl 6-amino-2-hydroxy-2-methyl-4-(3-nitrophenyl)1,2,3,4-tetrahydropyridine-3,5-dicarboxylate	

Table. 2: The FT-IR spectral data for Azelnidipine, stage and impurities.

Impurity	FT-IR stretching
Impurity-1	2957 Aliphatic C-H stretching; 1745,1718 C=O stretching; 1438,1408,1362,1321 Aliphatic C-H bending; 1270,1153,1041 C=O stretching.
Stage-I	3121,3088 Aromatic C-H stretching; 2987,2932,2877 Aliphatic C-H stretching; 1722 C=O stretching; 1664,1630 Aromatic C=C stretching; 1534 Aromatic $N(=O)_2$ Asymmetric stretching; 1466,1446,1388,1378 Aliphatic C-H bending; 1352 Aromatic $N(=O)_2$ symmetric stretching; 1295,1227 C-N stretching; 1212,1101,1039 C-(C=O)-O stretching; 826,812,736,678 Aromatic C-H bending
Impurity-2	3489 O-H stretching; 3071 Aromatic C-H stretching; 2984,2936,2879 Aliphatic C-H stretching; 1730,1716,1699 C=O stretching; 1533,1467664,1630 Aromatic C=C stretching; 1533 Aromatic $N(=O)_2$ Asymmetric stretching; 1467,1377 Aliphatic C-H bending; 1353 Aromatic $N(=O)_2$ symmetric stretching; 1305,1248 C-N stretching; 1199,1171,1107 C-(C=O)-O stretching; 828,816,737 Aromatic C-H bending

Impurity-3	3092,3037 Aromatic C-H stretching; 2987 Aliphatic C-H stretching; 1729 C=O stretching; 1662,1629 Aromatic C=C stretching; 1528 Aromatic $N(=O)_2$ Asymmetric stretching; 1447,1400,1384 Aliphatic C-H bending; 1351 Aromatic $N(=O)_2$ symmetric stretching; 1299,1247,1226 C-N stretching; 1211,1103, 1046, 1020 C-(C=O)-O stretching; 856, 822,813,735 Aromatic C-H bending
Impurity-4	3075 Aromatic C-H stretching; 2960,2927,2868 Aliphatic C-H stretching; 1722 C=O stretching; 1652,1621 Aromatic C=C stretching; 1529 Aromatic $N(=O)_2$ Asymmetric stretching; 1444,1390 Aliphatic C-H bending; 1356 Aromatic $N(=O)_2$ symmetric stretching; 1315,1252,1237 C-N stretching; 1216,1090, 1047 C-(C=O)-O stretching; 840,814,739 Aromatic C-H bending
Impurity-5	3023 Aromatic C-H stretching; 2983,2917,2849 Aliphatic C-H stretching; 1723 C=O stretching; 1676,1605 Aromatic C=C stretching; 1527 Aromatic $N(=O)_2$ Asymmetric stretching; 1473,1466,1376 Aliphatic C-H bending; 1346 Aromatic $N(=O)_2$ symmetric stretching; 1242 C-N stretching; 1217,1105, 1047 C-(C=O)-O stretching; 857,826,756 Aromatic C-H bending
Impurity-6	3112,3088,3051 Aromatic C-H stretching; 2986,2938 Aliphatic C-H stretching; 1720,1701 C=O stretching; 1623,1601 Aromatic C=C stretching; 1518 Aromatic $N(=O)_2$ Asymmetric stretching; 1462,1453,1422,1376 Aliphatic C-H bending; 1347 Aromatic $N(=O)_2$ symmetric stretching; 1255 C-N stretching; 1203,1194,1183,1105 C-(C=O)-O stretching; 855,765 Aromatic C-H bending
Azelnidipine	3448, 3321 N-H Stretching; 3085,3062,3027 Aromatic C-H stretching; 2977,2935,2842 Aliphatic C-H stretching; 1682,1651 C=O stretching; 1523 Aromatic $N(=O)_2$ Asymmetric stretching; 1489 Aromatic C=C stretching; 1453,1386 Aliphatic C-H bending; 1347 Aromatic $N(=O)_2$ symmetric stretching; 1320, 1246,1226,1103 C-N stretching; 753,743,704 Aromatic C-H bending
Impurity-7	3436 N-H Stretching; 3026 Aromatic C-H stretching; 2993,2857 Aliphatic C-H stretching; 1743,1676 C=O stretching; 1527 Aromatic $N(=O)_2$ Asymmetric stretching; 1490,1454 Aromatic C=C stretching; 1385 Aliphatic C-H bending; 1349 Aromatic $N(=O)_2$ symmetric stretching; 1310, 1288 C-N stretching; 1247,1216,1098,1046 Acetate (C=O)-O stretching; 742,706 Aromatic C-H bending

Impurity-8	3427 N-H Stretching; 3026 Aromatic C-H stretching; 2993,2857 Aliphatic C-H stretching; 1743,1676 C=O stretching; 1527 Aromatic $N(=O)_2$ Asymmetric stretching; 1490,1454 Aromatic C=C stretching; 1385 Aliphatic C-H bending; 1349 Aromatic $N(=O)_2$ symmetric stretching; 1310, 1288 C-N stretching; 1247,1216,1098,1046 Acetate (C=O)-O stretching; 742,706 Aromatic C-H bending
Impurity-9	3435 N-H Stretching; 3027 Aromatic C-H stretching; 2978,2930,2852 Aliphatic C-H stretching; 1673,1614 C=O stretching; 1552,1491 Aromatic C=C stretching; 1529 Aromatic $N(=O)_2$ Asymmetric stretching; 1453 Aliphatic C-H bending; 1354,1347 Aromatic $N(=O)_2$ symmetric stretching; 1310, 1240 C-N stretching; 1104 C- (C=O)-O stretching; 746,705 Aromatic C-H bending

Table. 3: The NMR Chemical shifts in δ(ppm) of Azelnidipine Raw material and its impurity (Impurity-1).

	Methyl Aceto Acetate				Impurity-1			
Position[a]	^{1}H	δ(ppm)	^{13}C		Position[a]	^{1}H	δ(ppm)	^{13}C
1	3H	2.17, s	25.96		1	3H	2.28, s	20.62
2	-	-	201.45		2	-	-	169.03
3	2H	3.61, s	49.31		3	1H	5.94, s	101.36
4	-	-	167.67		4	-	-	181.01
5	3H	3.63, s	51.71		5	-	-	99.78
-	-	-	-		6	-	-	161.13
-	-	-	-		7	-	-	205.17
-	-	-	-		8	3H	2.67, s	29.98
-	-	-	-		OH	1H	16.70, s	-

[a]Refer Table.1 for structures with numbering. s-singlet.

Table. 4: The ^{1}H NMR chemical shifts in δ (ppm) of Azelnidipine stage-I and its impurities (impurity-2 to 6).

Position[a]	^{1}H	Stage-I	Impurity-2	Impurity-3	Impurity-4	Impurity-5	Impurity-6
1	1H	8.31-8.38, m	8.25, br	8.39, t(1.8)	8.38, t(1.8)	-	8.26, d(8.7)[b]
2	-	-	-	-	-	7.44, d(7.5)[b]	7.69, d(9.0)[b]
3	1H	8.31-8.38, m	8.10-8.13, m	8.31-8.34, m	8.31-8.34, m	7.69-7.75 m	-
4	1H	7.32-7.84, m	7.62, t(8.0)[b]	7.80, t(8.0)[b]	7.80, t(8.0)[b]	7.81-7.86, m	7.69, d(9.0)[b]
5	1H	7.92-7.97, m	7.72-7.76, m	7.92-7.95, m	7.89-7.91, m	8.23-8.26, m	8.26, d(8.7)[b]
7	1H	7.92-7.97, m	3.97, t(12.0)[b]	7.99, s	8.02, s	8.20, s	7.72, s
8	-	-	3.43, d(11.7)[b]	-	-	-	-
10	3H	2.47,s	1.28, s	2.48, s	2.49, s	2.44, s	2.38, s
12	1H	5.09-5.17, m	4.67-4.75, m	4.24-4.31, q(7.2)[b]	3.79, s	4.79-4.87, m	5.04-5.12, m
13	3H	1.21, d(6.3)[b]	0.97, d(6.3)[b]	1.20, t(7.2)[b]	-	0.95, d(6.3)[b]	1.28, d(6.0)[b]

14	3H	1.21, d(6.3)[b]	1.02, d(6.3)[b]	-	-	0.95, d(6.3)[b]	1.28, d(6.0)[b]
15Ha	1H	-	2.37, d(13.5)[b]	-	-	-	-
15Hb	1H	-	2.94, d(13.5)[b]	-	-	-	-
17	1H	-	4.07, d(12.6)[b]	-	-	-	-
19	1H	-	4.57-4.65, m	-	-	-	-
20	3H	-	0.73, d(6.3)[b]	-	-	-	-
21	3H	-	0.88, d(6.0)[b]	-	-	-	-

[a]Refer Table.1, Fig.1 and Fig.2 for structures with numbering. s-singlet; d-doublet; t-triplet; dd-double doublet; q-quartet, m-multiplet; [b] ^{1}H-^{1}H Coupling constants.

Table. 5: The ^{13}C NMR chemical shifts in δ (ppm) of Azelnidipine stage-I and its impurities (impurity-2 to 6).

Position[a]	stage-I	Impurity-2	Impurity-3	Impurity-4	Impurity-5	Impurity-6
1	124.99	122.97	125.07	125.14	146.77	130.61
2	147.96	147.49	148.00	148.06	124.85	123.90
3	123.22	122.19	123.49	123.68	129.71	147.87
4	130.66	129.76	130.74	130.84	134.37	123.90
5	135.50	135.81	135.30	135.06	130.62	130.61
6	136.64	142.38	134.49	134.41	130.14	139.13
7	138.68	43.90	139.12	139.45	140.83	137.51
8	134.56	55.61	136.40	136.14	136.34	137.43
9	195.82	72.62	195.91	195.94	195.08	202.07
10	26.10	28.12	26.09	26.08	26.61	30.93
11	165.95	169.78	166.48	167.02	164.74	162.89
12	69.33	67.71	61.51	52.58	68.68	69.42
13	21.18	21.20	13.67	-	20.95	21.37
14	21.18	21.24	-	-	20.95	21.37
15	-	54.22	-	-	-	-
16	-	202.76	-	-	-	-
17	-	61.36	-	-	-	-
18	-	167.42	-	-	-	-
19	-	67.11	-	-	-	-
20	-	21.04	-	-	-	-
21	-	21.18	-	-	-	-

[a]Refer Table 1 for structures with numbering

Table. 6: The ^{1}H NMR chemical shifts in δ (ppm) of Azelnidipine and its impurities (impurity-7 to 9).

Position	^{1}H	Azelnidipine	Impurity-7	Impurity-8	Impurity-9
1	1H	8.05-8.10, m	7.90, br	8.22-8.25, m	-
2	-	-	-	-	7.15-7.62, m
3	1H	8.05-8.10, m	7.69, d(6.6)	8.00, br	7.15-7.62, m
4	1H	7.56-7.64, m	7.13.7.26, m	7.61-7.64, m	7.15-7.62, m
5	1H	7.56-7.64, m	7.50, d(7.8)	7.61-7.64, m	7.15-7.62, m
6	-	-	-	-	-
7	1H	4.74-4.87, m	4.72-4.80, m	4.19, s	4.17-4.83, m
8	-	-	-	2.54, s	
13	1H	4.74-4.87, m	4.98-5.01,m	4.74-4.81, m	4.17-4.83, m
14Ha	1H	2.86-2.90, m	2.30-2.32, m	2.27-2.33, m	2.66-2.71, m
14Hb	1H	3.45, t(6.9)		3.18-3.22, m	3.35-3.40, m
14'Ha	1H	2.27, br	4.21-4.23, m	1.65-1.67, m	2.57, t(7.1)
14'Hb	1H	3.27, br		3.00-3.05, m	2.83, t(6.8)
15	1H	4.17, br	4.50, s	3.74, s	4.34, s
17,21	2H	7.14-7.38, m	7.13-7.26, m	7.13-7.30, m	7.15-7.62, m
17',21'	2H	7.14-7.38, m	7.13-7.26, m	7.13-7.30, m	7.15-7.62, m
18,20	2H	7.14-7.38, m	7.13-7.26, m	7.13-7.30, m	7.15-7.62, m
18',20'	2H	7.14-7.38, m	7.13-7.26, m	7.13-7.30, m	7.15-7.62, m
19,19'	2H	7.14-7.38, m	7.13-7.26, m	7.13-7.30, m	7.15-7.62, m
22	3H	2.27,s	2.24, s	1.37, s	2.24, s
24	1H	4.74-4.87, m	4.72-4.80, m	4.60-4.64, m	4.71-4.83, m

25,25'	6H	0.99, 1.88, d(6.0),d(6.3)	0.95, 1.15, d(6.3),d(6.3)	0.83, 1.00 d(6.3),d(6.3)	0.90, 1.13 d(6.3),d(6.3)
27	-	-	2.02, s	-	
NH_2	2H	6.75, br	6.79, br	6.79, br	6.77-6.85, br
NH	1H	8.82, s	8.79,s	7.07,s	8.79,s
NH'	1H	-	7.13-7.26, m	-	
OH	1H	-	-	5.59, s	

[a]Refer Table 1 for structures with numbering. s-singlet; d-doublet; t-triplet; dd-double of doublet, m-multiplet; [b1]H-[1]H Coupling constants.

Table. 7: The ^{13}C NMR chemical shifts in δ (ppm) of Azelnidipine and its impurities (impurity-7 to 9).

Position	Azelnidipine	Impurity-7	Impurity-8	Impurity-9
1	122.49	121.97	122.68	147.00
2	151.74	151.56	151.04	123.84
3	120.69	120.44	120.78	126.78
4	129.54	128.86	129.41	130.77
5	134.56	134.19	134.65	133.22
6	146.93	146.88	147.29	144.18
7	38.82	38.67	39.50	32.77
8	102.78	102.94	58.71*	103.38
9	145.31	145.22	77.46	144.94
10	151.79	151.86	156.93	152.20
11	76.26	76.37	73.13	77.47
12	165.87	165.78	166.85	166.11
13	61.38	68.49	67.28	61.67

14	59.32	46.92	58.48	59.32
14'	60.18	63.41	63.36	59.51
15	77.30	65.89	77.11	76.48
16	142.11	144.02	142.04	142.33
16'	142.23	144.05	142.13	142.75
17,21	128.42	128.17	128.38	128.31
17',21'	128.42	128.17	128.38	128.36
18,20	126.95	126.71	126.80	127.01
18',20'	126.95	126.75	126.80	127.06
19,19'	127.06	126.66	127.02	126.95,126.84
22	18.63	18.55	27.43	18.72
23	167.18	167.03	169.58	167.50
24	66.33	66.18	59.99	66.22
25	21.45	21.40	21.26	21.18
25'	21.81	21.77	21.32	21.46
26	-	170.09	-	-
27	-	20.58	-	-

[a]Refer Table 1 for structures with numbering.

4.0 ACKNOWLEDGMENTS

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5.0 LITERATURE REFERENCES

- Oizumi, K.; Nishino, H.; Koike, H.; Sada, T.; Miyamoto, M.; Kimura, T.; Antihypertensive effects of CS-905, a novel dihydropyridine $Ca2^{+}$ channel blocker. Jpn J Pharmacol, 51, 1989, 57–64.
- Vasundhara, K.; Sandeepkumar.; and Sandhya, L.; Sitasawad.; Azelnidipine prevents cardiac dysfunction instreptozotocin-diabetic rats by reducing intracellular calcium accumulation, oxidative stress and apoptosis, Cardiovascular Diabetology, 10(97), 2011, 1-12.
- Sho-ichi, Y.; Yosuke, I.; Kazuo, N.; and Tsutomu, I.; Azelnidipine, A Newly Developed Long-Acting Calcium Antagonist, Inhibits Tumor Necrosis Factor-α-InducedInterleukin-8 Expression in Endothelial Cells through its Anti-Oxidative Properties, J Cardiovasc Pharmacol, 43, 2004; 724–730.
- Shinomiya, K.; Mizushige, K.; Fukunaga, M.; Masugata, H.; Ohmori, K.; Kohno, M.; and Senda, S.; Antioxidant Effect of a New Calcium Antagonist, Azelnidipine, in Cultured Human Arterial Endothelial Cells, The Journal of International Medical Research, 32, 2004, 170 – 175.
- International Conference on Harmonization (ICH) Guideline, Impurities in New Drug Substances Q3A (R2), October 25, 2006.
- International Conference on Harmonization (ICH) guidelines, validation of analytical procedures: Text and Methodology Q2 (R1), November 2005.
- Iwanami, M.; Shibanuma, T.; Fujimoto, M.; Kawai, R.; Tamazawa, K.; Takenaka, T.; Takahashi, K.; and Murakami, M.; Synthesis of new water-soluble dihydropyridine vasodilators, Chem. Pharm. Bull. (Tokyo), 27(6), 1979, 1426-1440.
- Meguro, K.; Aizawa, M.; Sohda, T.; Kawamatsu, Y.; and Nagaoka, A.; New 1-4-Dihydropyridine Derivatives with Potent and Long-Lasting Hypotensive Effect, Chem.Pharm. Bull. (Tokyo), 33 (9), 1985, 3787-3797.
- Morita, I.; Haruta, Y.; Tomita, T.; Tsuda, M.; Kandori, K.; Kise, M.; and Kimura, K.; Chem. Pharm. Bull. (Tokyo), Syntheses and antihypertensive activities of 1, 4-dihydropyridine-5-phosphonate derivatives. III, 35 (12), 1987, 4819-4828.
- Jones, G.; R. Adams, R.; Blatt, A.H.; Boekelheide, V.; Cairns, T.L.; Cram, D.J.; and House, H.O.; Organic Reactions: The Knoevenagel Condensation, 15, John Wiley & Sons, Inc., New York, 1967, 204-2012.
- Boese Jr, A.B.; Diketene A New Industrial Chemical, Ind. Eng. Chem., 32 (1), 1940, 16–22.
- Sudarshanrao, K.; K. Nageswararao, K.; Chavan, B.; Muralikrishna, P.; and Jayashree, A.; Identification and synthesis of impurities formed during preparation of Azelnidipine, Asian Journal of Chemistry, 26(15), 2015, 4675-4678.
- Dong, Li.; Min Wang.; Caiqin, Y.; Jing Wang, and Guodong, R.; Solid state Characterization and Analysis of Stability in Azelnidipine polymorphs, Chem.Pharm.Bull, 60 (8), 2012, 995-1002.

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