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POTENTIAL PROCESS RELATED SUBSTANCE OF ATORVASTATIN CALCIUM: IDENTIFICATION, SYNTHESIS AND SPECTRAL CHARACTERIZATION INCLUDING CONTROL STRATEGY

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ABSTRACT

HPLC analysis of Atorvastatin Calcium showed an unknown compound peak, the sample was also analyzed by LCMS and the corresponding peak was identified at m/z 854.06. The pure compound was prepared individually and characterized. The compound structure was assigned as tert-butyl 2-((4*R*,6*R*)-6-(2-(2-((4*R*,6*R*)-6-(2-[2-(4-fluoro-phenyl)-5-isopropyl-3-phenyl-4-phenylcarbamoyl-pyrrol-1-yl]-ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetamido)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate.

Keywords: Atorvastatin Calcium, Related Substance, Paal–Knorr Condensation, EP Impurity-F, Control Strategy.
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INTRODUCTION

Atorvastatin Calcium (**1**), is a medication used to lower cholesterol levels to help prevent heart disease,[1-5] HMG-CoA reductase inhibitor,[6-7]strokes,[8-9] and lipid-lowering.[10-14] Atorvastatin calcium is also used to treat the prevention of cardiovascular disease with atorvastatin in type 2 diabetes[15-16] and glycaemia[17,] diabetic dyslipidemia,[18] and cerebrovascular prevention.[19]The ICH recommended guidelines that the acceptable level for an unknown or known related substance (impurity) is less than 0.10 % and 0.15 respectively in a drug substance. Formation of the related substance during the process is inevitable in the drug substance. Hence, the identification and characterization of the related substance present in the drug substance is a must. A lot of research has been done on Atorvastatin Calcium and many impurities are identified, isolated, synthesized, characterized, and reported in the literature.[20-23]During the synthesis and process development of Atorvastatin Calcium (Scheme-1), in addition to known impurities, one compound was detected by simple reverse-phase HPLC. This compound is identified as protected EP impurity-F (**2**) of atorvastatin and is the precursor for EP impurity-F (**3**). The literature survey indicates that the synthesis of compound **2** is not available. The present work consists of the identification, synthesis, and characterization of compound **2**.

EXPERIMENTAL

Materials

The chemicals, intermediates, reagents, and solvents are commercially available and of the highest quality. Ultrapure water was used in all the experiments.

Instrumentation

^{1}H and ^{13}C-NMR spectra were recorded on an "Advance Neo Nano Bay 400 MHz FT NMR spectrometer" and the mass spectra were recorded on "ABSCIEX-API2000".

Method-1

Procedure for the Synthesis of Sodium Salt of Protected Atorvastatin 9

The NaOH (23 mmol) was added slowly to a solution of **7** (7.7 mmol), MeOH (15 mL), and THF (15 mL). The temperature rose to 40 to 45°C and maintained for 18 h. The progress was monitored by thin-

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layer chromatography. The solvent was distilled off and the obtained residue was treated with hexane to isolate sodium salt of protected atorvastatin **9** as a solid in 4.5 g (95%). ^{1}H-NMR (Dimethyl sulfoxide-d_6, 400 MHz): δ 9.96 (s, 1H), 7.55-7.53 (m, 2H), 7.24-7.21 (m, 6H), 7.19-6.96 (m, 6H), 4.07-3.94 (m, 1H), 3.92-3.81 (m, 1H), 3.80-3.71 (m, 2H), 3.23-3.21 (t, 1H), 2.12-2.07 (dd, 1H), 1.86-1.80 (m, 1H), 1.51-1.48 (bs, 3H), 1.36-1.29 (m, 9H), 1.16 (s, 3H), 0.85-0.77 (m, 1H). ^{13}C-NMR (Dimethyl sulfoxide-d_6, 400 MHz): δ 19.89, 22.08, 22.41, 25.65, 29.99, 36.52, 37.97, 45.55, 66.25, 67.34, 97.64, 115.26, 115.47, 117.64, 119.47, 120.66, 122.95, 125.38, 127.29-127.63, 128.42, 128.69, 129.24, 133.35, 133.43, 134.91, 135.90, 139.53, 160.40, 162.83, 166.18, 166.65, 174.05. Mass m/z 642.9 $(M+Na)^+$.

Procedure for the synthesis of protected EP Impurity-F (2)

The compound **9** (8 mmol), DiPEA (32 mmol) were taken in DMF (50 mL), and HOBt (16 mmol), EDC HCl (12 mmol) were added slowly at 0 to 10°C followed by **5** (9 mmol). The temperature of the reaction mass rose to 20 to 30°C and was maintained for 16 h. Reaction progress was monitored by TLC. The reaction mass was poured into ice-cold water (200 mL) and stirred at room temperature for 2 h. The filtered solid was dried at 50°C to isolate compound **2** in 6 g (87%). The compound was purified by column chromatography (methanol containing chloroform (95:5) as eluent) to obtain pure compound **2** (2.1 g).^{1}H-NMR (Dimethyl sulfoxide-d_6, 400 MHz): δ 9.78 (s, 1H), 7.73 (t, 1H), 7.52-7.50 (m, 2H), 7.27-7.17 (m, 6H), 7.10-7.07 (m, 4H), 7.02-6.96 (m, 2H), 4.19 -4.09 (m, 2H), 3.96 -3.75 (m, 4H), 3.26-3.19 (m, 1H), 3.09-3.03 (m, 2H), 2.39-2.33 (m, 1H), 2.24-2.16 (m, 2H), 2.08-2.03 (m, 1H), 1.54-1.46 (m, 5H), 1.39-1.29 (m, 19H), 1.29 (s, 3H), 1.23 (s, 3H), 1.17 (s, 3H), 1.08-0.99 (q, 1H), 0.94-0.85 (q,1H) ,^{13}C-NMR (Dimethyl sulfoxide-d_6, 400 MHz): δ 19.57, 9.72, 22.09, 22.33, 25.58 , 27.70, 29.79, 29.91, 34.61, 35.61, 35.69, 37.81, 42.16, 42.46, 65.88, 65.96, 66.17, 79.67, 97.93, 97.99, 115.21, 115.42 , 117.51, 119.37, 120.56, 122.93, 125.35, 127.28, 127.60, 128.39, 128.59, 129.11, 133.34, 133.42, 134.81, 135.96, 139.41, 160.39, 162.83, 166.05, 168.95, 169.55. Mass m/z 876.4 $(M+Na)^+$.

Method-2

Procedure for the synthesis of *N*-benzyl 2-((4*R*,6*R*)-6-((tert-butoxycarbonyl)methyl)-2,2-dimethyl-1,3-dioxan--4-yl)ethyl Carbamate (10)

To a mixture of **5** (18 mmol), acetone (6.25 mL), water (12.5mL), TBAB (0.44 mmol) and potassium carbonate (18 mmol), benzyl chloroformate (26.6 mmol) was added at 25 to 35°C. The mixture was stirred at rt for 16 h. After completion of the reaction, compound **10** was isolated from the aqueous layer as a solid in 7.2 g (96.5%). ^{1}H-NMR (Dimethyl sulfoxide-d_6, 400 MHz): δ 7.38-7.29 (m, 5H), 7.19 (t, 1H), 5.02 (s, 2H), 4.19-4.15 (m, 1H), 3.92-3.86 (m, 1H), 3.09-3.04 (m, 2H), 2.37-2.32 (m, 1H), 2.25-2.19 (m, 1H),1.54-1.49 (m, 3H),1.39-1.36 (m, 12H),1.24 (s, 3H), 1.09-1.00 (m, 1H). ^{13}C-NMR (Dimethyl sulfoxide-d_6, 400 MHz): δ 19.52, 27.69, 29.89, 35.73, 35.99, 36.52, 42.14, 65.09, 65.92, 66.14, 79.62, 98.00, 127.68, 128.27, 137.26, 156.03, 169.54. Mass m/z 430.5 $(M+Na)^+$.

Procedure for the synthesis of sodium salt of *N*-benzyl 2-((4*R*, 6*R*)-6-((hydroxycarbonyl)methyl)-2, 2-dimethyl-1,3--dioxan-4-yl)ethyl carbamate (11)

To a solution of **10** (24.5 mmol) in MeOH (30 mL) and THF (30 mL), NaOH (0.17 mol) was added. Raised the temperature to 45°C and maintained it for 18 h. The solvent was distilled off and hexane was added to the obtained residue to get 7.5 g (82 %) of Cbz-protected amino acid salt **11**. ^{1}H-NMR (Dimethyl sulfoxide-d_6, 400 MHz): δ 7.38 – 7.28 (m, 5H), 7.21 (t, 1H), 5.06-5.01(s, 2H), 4.21-4.17 (m, 1H), 3.92-3.87 (m, 1H), 3.06-3.03 (m, 2H), 2.34- 2.25 (m, 2H), 1.58-1.48 (m, 3H), 1.36 (s, 3H), 1.24 (s, 3H), 1.08-0.99 (m, 1H),^{13}C-NMR (Dimethyl sulfoxide-d_6, 400 MHz): δ 19.67, 29.96, 35.98, 36.56, 38.87, 40.12, 41.39, 65.14, 65.89, 66.12, 98.07, 127.34, 127.73, 128.34, 137.28, 156.07, 172.05. Mass m/z 374.1 $(M+Na)^+$.

Procedure for the synthesis of tert-butyl 2-((4*R*,6*R*)-6-(2-(2-((4*R*,6*R*)-6-(2-*N*-benzyloxycarbonylaminoethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetamido)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (12)

To a mixture of **5** (18 mmol), DiPEA (35.5 mmol) and DMF (50 mL), EDC HCl (21 mmol) and HOBt (25.5 mmol) were added at 0 to 10°C. The compound **11** (11.3 mmol) was added slowly, raised the temperature 20 to 30°C, and maintained for 16 h. Ice cold water (200 mL) was added and extracted with

ethyl acetate (50 mL x 2). The solvent was distilled off to obtain Cbz-protected diamide **12**in 5 g (45%). ^{1}H-NMR (Dimethyl sulfoxide-d_6, 400 MHz): δ 7.76 (t, 1H), 7.38-7.28 (m, 5H), 7.19 (t, 1H), 5.05-5.00 (s, 2H), 4.19-4.16 (m, 2H), 3.89-3.85 (m, 2H), 3.10-3.04 (m, 4H), 2.38-2.33 (m, 1H), 2.25-2.18 (m, 2H), 2.13-2.08 (m, 1H), 1.55-1.46 (m, 6H), 1.39 -1.35 (m, 15H), 1.24 (s, 6H), 1.09-0.97 (m, 2H). ^{13}C-NMR (Dimethyl sulfoxide-d_6, 400 MHz): δ 29.89, 29.96, 34.59, 35.68, 36.01, 36.07, 36.49, 42.15, 42.66, 65.07, 65.95, 66.11, 66.16, 79.65, 97.98, 127.67, 128.28, 137.23, 155.99, 169.03, 169. 53. Mass m/z: 607.1 $(M+H)^+$.

Procedure for the synthesis of amino ester side chain 8

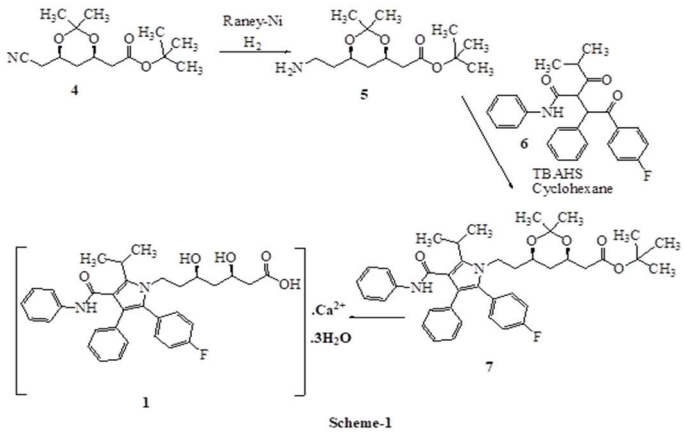
The 5 % palladium carbon (0.2 g) was added to asolution of Cbz-protected diamide **12** (3.3 mmol) in methanol (10 mL) under a nitrogen atmosphere. Then the hydrogen pressure was applied with a balloon and maintained at room temperature for 4 h. Palladium carbon was filtered off. Distilled offthe filtrate to obtain amino ester**8** (1.19 g, 77%). ^{1}H-NMR (Dimethyl sulfoxide-d_6, 400 MHz): δ 7.75 (t, 1H), 4.19-4.16 (m, 2H), 3.96-3.89 (m, 2H), 3.09-3.04 (m, 2H), 2.56-2.50 (m, 2H), 2.38-2.33 (m, 2H), 2.25-2.18 (m, 2H), 2.13-2.08 (m, 1H), 1.55-1.44 (m, 5H), 1.42-1.36 (m, 17H), 1.28 (s, 6H), 1.08-0.90 (m, 2H). ^{13}C-NMR (Dimethyl sulfoxide-d_6, 400 MHz): δ 20.01, 20.23, 28.15, 30.37, 30.47, 30.84, 35.09, 36.10, 36.19, 36.79, 37.97, 40.53, 42.66, 43.20, 49.02, 66.45, 66.66, 66.94, 80.15, 98.39 98.49, 125.34, 169.65, 170.05. Mass m/z: 473.0 $(M+H)^+$.

Alternative procedure for the synthesis of protected EP impurity-F(2)

Pivalic acid (24 mmol), morphine (11 mmol), and TBAHS (1.8 mmol) were added to the suspension of **6** (12 mmol) in cyclohexane (50 mL) at ambient temperature. The temperature rose to 50 to 60°C,and a solution of compound **8** (12 mmol) in cyclohexane (10mL) was added and refluxed for 34 h. The reaction mass was quenched with $NaHCO_3$ solution (25 mL) at 55 to 60°C. The organic layer was distilled off to obtain compound **2** (4.6 g, 45%).

RESULTS AND DISCUSSION

Compound **1** is synthesized by the reduction of the cyano group of tert-butyl 2-((4*R*,6*R*)-6-(cyanomethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (**4**) in presence of Raney-Nickel, Paal–Knorr condensation of the resultant tert-butyl 2-((4*R*,6*R*)-6-(2-aminoethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (**5**) with 2-(2-(4-fluorophenyl)-2-oxo-1-phenylethyl)-4-methyl-3-oxo-*N*-phenylpentanamide (**6**) in cyclohexane in presence of tetra-butyl-ammonium-hydrogen-sulfate (TBAHS) to yield tert-butyl 2-((4*R*,6*R*)-6-(2-(3-(phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (**7**) followed by deprotection and sanctification (Scheme-1).



Scheme-1

Possibilities of formation of impurity-F

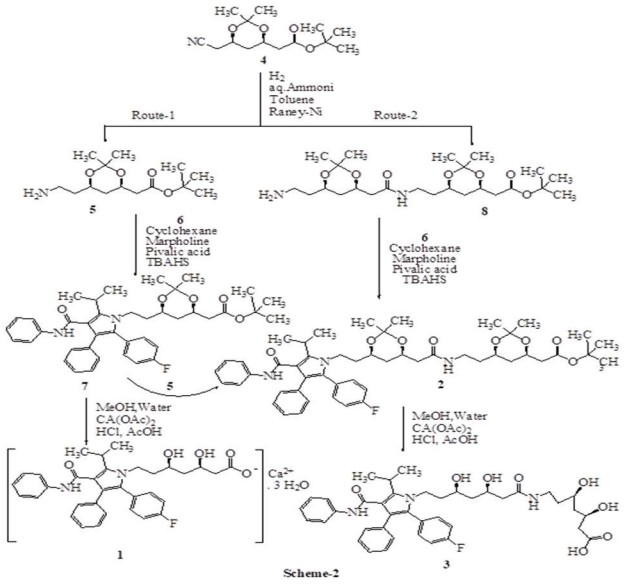
Two possible routes have been identified for the formation of Impurity-F.

Route-1

Condensation of ester derivative **7** with amino derivative **5** to yield diamide **2** followed by deprotection to afford compound **3**i.e. EP impurity-F (**3,** Scheme-2).

Route-2

Self-condensation of amine **5** yields amino ester **8**. The Paal–Knorr condensation of compound **8** with diketone **6** in cyclohexane containing morpholine, pivalic acid, and TBAHS to afford key compound **2** followed by deprotection to yield EP impurity-F (**3,** Scheme-2**)**.



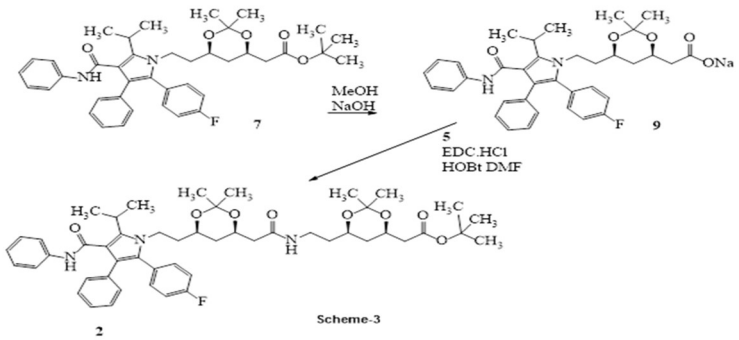
Scheme-2

Synthesis of Compound 2

The related substance **2** has been synthesized by two methods.

Method-1

The ester compound **7** was reacted with NaOH in a mixture of MeOH and THF at 40 to 45°C for 18 h to get sodium salt of protected atorvastatin **9**. The resultant **9** was condensed with amine **5** in DMF containing EDC HCl, HOBt, and DiPEA to isolate diamide **2** (Scheme-3).



Scheme-3

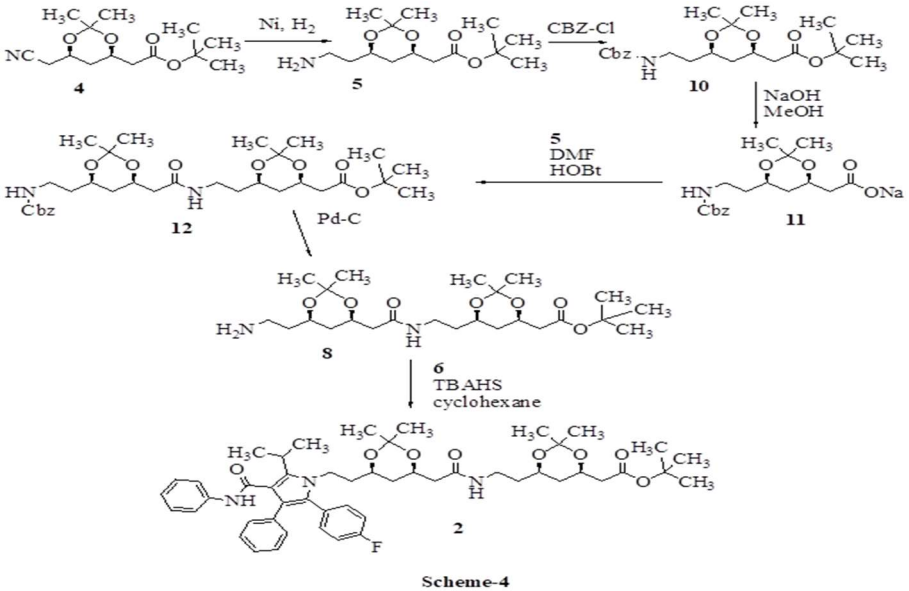
Method-2

The benzyl chloroformate was reacted with compound **5** in aq. acetone in the presence of K_2CO_3 to protect amino group of **5** to generate **10**, hydrolysis of ester group in basic medium to corresponding carboxylic acid salt **11**, then coupling with **5** in DMF in the presence of HOBt and DiPEA to get amide compound **12**, deprotecting the amine with Pd-C/H_2 in methanol to compound **8**, the Paal–Knorr condensation of the amine group of **8** with diketone **6** in cyclohexane containing pivalic acid, morphine and TBAHS at room temperature to get key diamide compound **2** (Scheme-4).

Control Strategy of Compound 2 in Atorvastatin

1. In the reduction of compound **4** to amine **5,** aprotic solvents like toluene should be used instead of protic solvents like methanol, distilling off the toluene during workup should be below 55ºC and compound **5** should be protected from the acids like HCl gas as it is sensitive.

2. Conversion of compound **5** to **7**, mole ration of compound **6** should be more than compound **5,** and usage of the TBAHS is at least in 0.15 equivalents.



Scheme-4

CONCLUSION

In conclusion, the present investigation provides identification, scope of formation, synthesis, and characterization of the diamide **2**. The present investigation also provides a control strategy of diamide **2** formation so that EP compound F also controls which have regulatory importance.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors significantly contributed to this manuscript, participated in reviewing and editing, and approved the final draft for publication. The authors' research profiles can be verified through their ORCID IDs listed below:

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