Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(4):251-270
(http://derpharmachemica.com/archive.html)

Identification, characterization and preparation of process-related impurities of the phenylquinoline derivative NK-104

Madhuresh K. Sethi*, Sanjay Mahajan, Bhairaiah Mara, Upendranath Veera, Anish Kumar, Anitha Nimmagadda, Purbita Chakraborty, Mujahid Sufi Ahmed and Rohit Shukla

R & D, Mylan Laboratories Ltd., Plot No. 31, 32, 33 and 34 A ANRICH Industrial Estate, Bollaram (Village), Jinnaram (Mandal), Medak (Dt) 502325, Andhra Pradesh, India

---

ABSTRACT

The related substances observed in Phenylquinoline derivative NK-104 (A lipid-lowering agent) were isolated, characterized and their proposed structures were confirmed by chemical synthesis.These related substances were assigned as [2-cyclopropyl-4(4-fluorophenyl)quinolin-3-yl]methanol,(4R,6S)-6-{(E)-2-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]ethenyl]-4-hydroxytetrahydro-2H-pyran-2-one,Calcium bis{(3R,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-3(R)-hydroxy-5-oxohept-6-enoate, {(3R, 5S, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-3, 5-dihydroxy-N-(1-phenylethyl)hept-6-enamide}, Calcium bis {(3R, 5S, 6E)-7-[2-cyclopropyl-4-(4-phenyl)-quinolin-3-yl]-3, 5-dihydroxyhept-6-enoate}, Calcium bis {(3R,5S)-7-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-3,5-dihydroxyheptanoate}, Calcium bis {(3S, 5R, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolin-3-yl]-3, 5-dihydroxyhept-6-enoate},Calcium bis {(3R, 5R, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolin-3-yl]-3,5-dihydroxyhept-6-enoate},(6S)-6-{(E)-2-[2-cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl]ethenyl}-5,6-dihydro-2H-pyran-2-one, (3S,4E,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl]-3-hydroxyhepta-4,6-dienoic acid, (3R,5S)-5-(6-cyclopropyl-10-fluoro-7,8-dihydrobenzo[k] phenanthridine-8-yl)-3,5-dihydroxypentanoic acid. Investigation for the cause of these impurities and their identification helped in improvingthe yield & qualityduring preparation of this drug which helps in providing this high quality drug at lower cost.

Keywords: Phenylquinoline• NK 104•Pitavastatin calcium• impurity profile• related substances• Enantiomers• Diastereoisomers

---

INTRODUCTION

NK-104 (Pitavastatin Calcium) is an anti-lipidemic agent belonging to the class of phenylquinolines group of organic compounds. It is a selective, potent and competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase which catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in cholesterol biosynthesis[1].Studies have shown that pitavastatin calcium lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver by increasing the number of hepatic Low Density Lipoprotein (LDL) receptors on the cell-surface for enhanced uptake and catabolism of LDL. Additionally, this drug inhibits the hepatic synthesis of Very Low Density Lipoprotein (VLDL), thereby reducing the total number of VLDL and LDL particles[2-3].

The HPLC analysis of Pitavastatin Calcium displayed four impurity peaks in the range of 0.03 to 0.15%levels along with the Pitavastatin peak. As per the guidelines recommended by ICH, the acceptable level for a known or unknown related compound (impurity) is less than 0.15 and 0.10 % respectively in a drug substance. In order to meet the stringent regulatory requirements, the impurities present in the drug substance must be identified and

characterized. Present work deals with the identification, synthesis and characterization of impurities/related substances of Piatavstatin Calcium. [4-9]

MATERIALS AND METHODS

2.1 Analytical

HPLC was carried out using Waters HPLC having 2487 UV detector with empower chromatography software. Column symmetry used is C18, 158× 4.6 mm, 5 µm with UV- Detector at 250 nm. Flow rate is maintained at 1.0 ml/min with injection volume of 10 µL using diluent Acetonitrile. The ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO on a Bruker Avance 300 spectrometer. The chemical shifts are reported in δ ppm relative to TMS (δ 0.00) and DMSO and D_2O as internal standards respectively. Electron Spray Ionization-Mass spectra (ESI-MS) of isolated compounds were measured using Agilent 1100 LC/MSD Trap SL instrument.

2.2 Chemicals

All the chemicals and reagents used were of commercial grade.

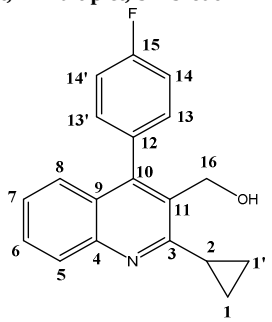
2.3 Synthesis

2.3.1 Preparation of [2-cyclopropyl-4(4-fluorophenyl)quinolin-3-yl]methanol impurity (Alcohol impurity of Pitavastatin calcium)[10]

A suspension of PTC aldehyhyde (10 g) in 100 ml tetrahydrofuran and 30 ml methanol was cooled to 0-10 ^{o}C. 1.6 g of sodium borohydride was then added, stirred for 2 hr at same temperature and extracted ethylacetate. The organic layer was washed with water and brine solution and then concentrated under reduced pressure and isolated the compound by adding 50 ml diisopropyl ether, filtered and dried to give [2-cyclopropyl-4(4-fluorophenyl)quinolin-3-yl] methanol (Alcohol impurity of Pitavastatin calcium) (6.0 g). HPLC – Alcohol impurity- 99.83 %, other impurities – 0.06 %, 0.11 %, Mass m/z = 294 (M + H), UV (λ nm) 238, 214, IR 3431 (O-H stretching), 3008 (Aromatic C-H stretching), 2898 (Aliphatic C-H stretching), 1605, 1579, 1514, 1496 (Aromatic C=C/C=N stretching), 1415 (Aliphatic C-H Bending), 1225, 1159, 1068, 1028, 1017 (C-F stretching), 842, 770 (Aromatic C-H stretching)

NMR (DMSO – d_6, 300 MHz)

Table 1: NMR data s- singlet, d- doublet, t- triplet, m-multiplet, br- broad ^{11}H- ^{1}H Coupling constant ^{13}C – ^{19}F Coupling constant



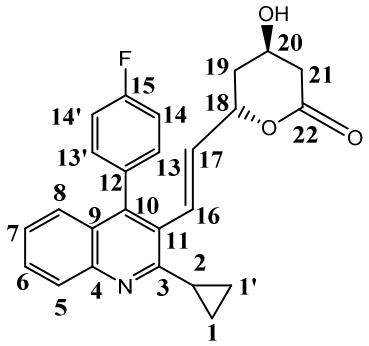
Position	1H	δ (ppm)	J (Hz)[1]	^{13}C, J(Hz)[2]	DEPT
1Ha, 1'Ha	2H	1.03-1.08	m	10.39	CH_2
1Hb, 1'Hb	2H	1.19-1.23	m		
2	1H	2.70-2.76	m	14.04	CH
3	-	-	-	162.86	-
4	-	-	-	146.39	-
5	1H	7.87	d (8.1)	126.04	-
6	1H	7.62 -7.68	m	128.36	CH
7	1H	7.21-7.24	dd (8.4, 0.9)	129.01	CH
8	1H	7.36-7.43	m	125.51	-
9	-	-	-	125.59	-
10	-	-	-	145.12	-
11	-	-	-	130.09	CH
12	-	-	-	132.34, d(3.4)	CH
13, 13'	2H	7.36-7.43	m	131.72, d(8.0)	-
14, 14'	2H	7.36-7.43	m	115.23, d(21.3)	-
15	-	-	-	161.89, d(242.9)	CH_3
16	2H	4.50	d(4.8)	57.82	CH_2
OH	1H	5.10	t(4.7)	-	-

2.3.2 Preparation of (4R,6S)-6-{(E)-2-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]ethenyl]-4-hydroxytetrahydro -2H-pyran-2-one (Lactone impurity of Pitavastatin calcium)[(11)]

To 500 ml ethyl acetate was added 20.0 g (S)-(-)-α-methyl benzyl amine salt of [(3R, 5S, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3, 5-dihydroxy-6-heptenoic acid], and to this 250 ml DM water was added. pH of the mixture was adjusted to 3.0 with aqueous hydrochloric acid solution. Reaction mixture is stirred and layers are separated. Washed the organic layer with water and brine solution, dried and concentrated under reduced pressure to give thick residue. To the residue 200 ml toluene was added and heated to the reflux for 5 hours using Dean stark apparatus. Then 200 ml n-heptane was added and cooled the solution to 0^{o}C, stirred, filtered, washed with toluene and dried the compound to get 5.0 g of (4R,6S)-6-{(E)-2-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]ethenyl]-4-hydroxytetrahydro-2H-pyran-2-one (Lactone impurity of Pitavastatin calcium). HPLC- 96.23 % other impurities – 2.79 %, 0.92 %, Mass m/z = 404 (M +H), UV (λ nm) 243, 205, 203, IR (cm -1) 3425 (O-H stretching), 3067, 3011 (Aromatic C-H stretching), 2953, 2922 (Aliphatic C-H stretching), 1733, 1712 (C=O stretching), 1602, 1568, 1512, 1489 (Aromatic C=C/C=N stretching), 1411, 1375 (Aliphatic C-H bending), 1360 (O-H bending), 1216 (C-F stretching), 1158, 1065, 1038 (C-O stretching), 834, 766 (Aromatic C-H bending)

NMR (DMSO – d_6, 300 MHz)

Table 2: NMR data d- doublet, dd- doublet of doublet, m-multiplet ^{11}H- ^{1}H Coupling constant ^{13}C – ^{19}F Coupling constant



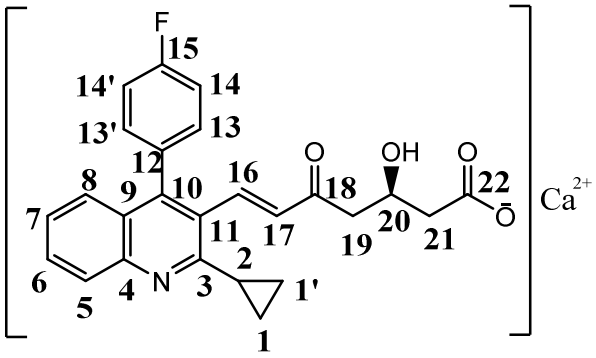
Position	1H	δ (ppm)	J (Hz)[1]	^{13}C, J(Hz)[2]	DEPT
1Ha, 1'Ha	2H	1.03-1.09	m	10.55, 10.61	CH_2
1Hb, 1'Hb	2H	1.20-1.24	m		
2	1H	2.30-2.47	m	15.34	CH
3	-	-	-	160.18	-
4	-	-	-	144.13	-
5	1H	7.88	d (8.4)	125.69	CH
6	1H	7.64 -7.69	m	129.02	CH
7	1H	7.28-7.44	m	128.73	CH
8	1H	7.28-7.44	m	128.36	CH
9	-	-	-	146.18	-
10	-	-	-	145.12	-
11	-	-	-	125.42	-
12	-	-	-	132.76, d(3.3)	-
13	1H	7.28-7.44	m	131.73, d(8.1)	CH
13'	1H	7.28-7.44	m	132.07, d(8.0)	CH
14	1H	7.28-7.44	m	115.23, d(21.3)	CH
14'	1H	7.28-7.44	m	115.20, d(21.3)	CH
15	-	-	-	161.63, d(243.2)	-
16	1H	6.68	d(15.9)	136.21	CH
17	1H	5.61-5.68	dd (6.6, 16.2)	126.83	CH
18	1H	5.04-5.11	m	75.53	CH
19	2H	1.52-1.58	m	38.51	CH_2
20	1H	4.00-4.04	m	61.02	CH
21Ha	1H	2.30-2.47	m	35.26	CH_2
21Hb	1H	2.58-2.65	dd(17.4, 4.5)		
22	-	-	-	169.65	-
OH	1H	5.10	t(4.7)	-	-

2.3.3 Preparation of 5- keto acid impurity

To a solution of 10 g of Methyl (E)-7-[2-cyclopropyl-4-(4-flurophenyl)-3-quinolinyl]-(3R)-3-hydroxy-5-oxo-6-heptenoate in ethyl alcohol (100 ml) and tetrahydrofuran (40 ml), was added solution sodium hydroxide (1.0 g) in water (40 ml). The reaction mixture was stirred at room temperature for 2 h. The reaction mass was concentrated under reduced pressure to get residue. Water (100 ml) and dichloromethane (100 ml) was added to the resulting

residue and was cooled to 10°C. pH is adjusted to 3.0 with ~ 35 % Aq. hydrochloric acid solution, stirred and the layers are separated. The organic layer is washed with brine solution, dried and concentrated to get residue. To the residue ethyl acetate and water was added and was cooled to 10°C. pH was adjusted to 9.0- 11.0 with ~ 4 % aqueous sodium hydroxide solution, stirred and the layers are separated. Washed the aqueous layer with ethyl acetate. To the aqueous layer added solution of calcium acetate (1.97 g) in water (50 ml). The suspension is stirred for 3h at room temperature. The mixture is filtered and washed with water, dried to give 7.0 of Calcium bis{(3R,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-3(R)-hydroxy-5-oxohept-6-enoate. HPLC- 96.30%, other impurities – 2.52 %, 0.49 %, 0.39 %, 0.13 %, MASS m/z = 420 (M +H), 418 (M – H),UV spectrum (λ nm) 275, 202, IR spectrum (cm^{-1}) 3411 (O-H stretching), 3067, 3006 (Aromatic C-H stretching), 2925 (Alipahtic C-H stretching), 1655 (C=O stretching), 1604, 1562, 1514, 1489 (Aromatic C=C/C=N stretching), 1412, 1325 (Aliphatic C-H bending), 1224 (C-F stretching), 1160, 1095, 1063 (C-O stretching), 843, 763 (Aromatic C-H bending)

Table 3: NMR data s- singlet, d- doublet, dd- doublet of doublet, t- triplet, m- multiplet, br- broad ^{11}H-^{1}H Coupling constants



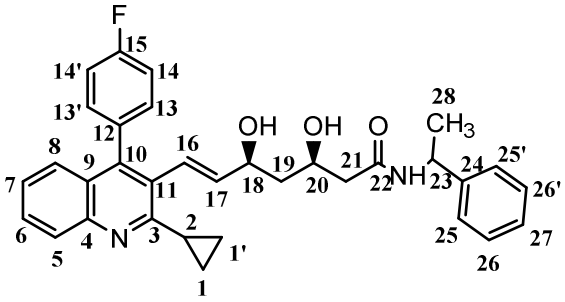
Position	1H	δ (ppm)	J (Hz)	^{13}C, J(Hz)[2]	DEPT
1Ha, 1'Ha	2H	1.06 -1.15	m	10.84	CH_2
1Hb, 1'Hb	2H	1.25	br		
2	1H	2.40- 2.46	m	15.93	CH
3	-	-	-	159.86	-
4	-	-	-	146.54	-
5	1H	7.89	d (8.4)	129.92	CH
6	1H	7.69	t(7.3)	128.48	CH
7	1H	7.30-7.46	m	126.17	CH
8	1H	7.30-7.46	m	126.00	CH
9	-	-	-	127.71	-
10	-	-	-	145.41	-
11	-	-	-	125.31	-
12	-	-	-	132.33, d(3.7)	-
13, 13'	2H	7.30-7.46	m	131.98, d(8.3)	CH
14, 14'	2H	7.30-7.46	m	115.50, d(21.4)	CH
15	-	-	-	161.90, d(243.8)	-
16	1H	7.56	d(16.5)	138.91	CH
17	1H	6.31	d (16.5)	144.53	CH
18	-	-	-	198.27	-
19	2H	2.55-2.57	m	47.96	CH_2
20	1H	4.07	br	65.08	CH
21Ha	2H	1.94-2.01	dd (15.2, 8.0)	43.78	CH_2
21Hb	1H	2.06-2.13	dd (15.5, 4.2)		
22	-	-	-	187.75	-
OH	1H	6.07	br	-	-

[2] ^{13}C-^{19}F Coupling constants

2.3.4 Preparation of {(3R, 5S, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-3, 5-dihydroxy-N-(1-phenyl ethyl) hept-6-enamide} Amide impurity of Pitavastatin calcium

To a solution of Pitavastatin Acid (5.0 g) and α-methyl benzyl amine (3.0 g) in dichloromethane (200 ml), Dicyclohexyl carbodiimde (10.0 g) was added, stirred for 20 -24 h at room temperature and filtered. The filtrate was concentrated and purified by preparative HPLC to yield {(3R, 5S, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl]-3, 5-dihydroxy-N-(1-phenylethyl)hept-6-enamide} (Amide impurity of Pitavastatin calcium), HPLC-96.20 %, 1.96 %, 0.75 %, 0.43 %, 0.11 %, MASS m/z = 525 (M + H), IR (cm-1) 3310 (N-H/O-H stretching), 3065, 3008 (Aromatic C-H stretching), 2930 (Alipahtic C-H stretching), 1648, 1605 (C=O stretching), 1542, 1513, 1490, 1449 (Aromatic C=C/C=N stretching), 1412 (Aliphatic C-H bending), 1386 (O-H bending), 1218 (C-F stretching), 1158, 1094, 1065, 1025 (C-O stretching), 834, 758, 700 (Aromatic C-H stretching), UV spectrum: (λ nm) 245, 222

Table 4: NMR data. s- singlet, d- doublet, dd- doublet of doublet, t- triplet, m- multiplet, br- broad ^{11}H-^{1}H Coupling constants ^{213}C-^{19}F Coupling constants

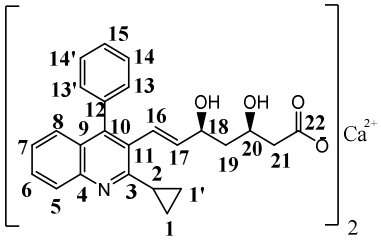


Position	1H	δ (ppm)	*J* (Hz)	^{13}C, J(Hz)^{2}	DEPT
1Ha, 1'Ha	2H	0.96 -1.15	m	10.69, 10.85	CH_2
1Hb, 1'Hb	2H	1.18 – 1.26	m		
2	1H	2.11- 2.24	m	15.41	CH
3	-	-	-	160.48	-
4	-	-	-	143.62	-
5	1H	7.86	d (8.4)	125.66	CH
6	1H	7.64	t(7.5)	128.82	CH
7	1H	7.16-7.41	m	128.35	CH
8	1H	7.16-7.41	m	125.61	CH
9	-	-	-	129.43	-
10	-	-	-	145.90	-
11	-	-	-	125.61	-
12	-	-	-	132.94, d(3.2)	-
13, 13'	2H	7.16-7.41	m	132.07, d(8.0)	CH
14, 14'	2H	7.16-7.41	m	115.26, d(21.2)	CH
15	-	-	-	161.61, d(237.2)	-
16	1H	6.47	d(16.2)	141.91	CH
17	1H	5.58-5.65	dd (16.2, 5.7)	125.90	CH
18	1H	4.11-4.15	m	68.75	CH
19	2H	1.18-1.26	m	43.48	CH_2
20	1H	3.80-3.81	m	65.27	CH
21	2H	2.11-2.24	m	44.50	CH_2
22	-	-	-	169.79	-
23	1H	4.89-5.01	m	22.56	CH
24	-	-	-	125.54	-
25, 25'	2H	7.16-7.41	m	128.15	CH
26, 26'	2H	7.16-7.41	m	125.90	CH
27	1H	7.16-7.41	m	123.44	CH
28	3H	1.32-1.40	m	47.59	CH_3
OH'	1H	4.70	d (4.5)	-	-
OH''	1H	4.85	d (4.2)	-	-
NH	1H	8.24	d (8.7)	-	-

2.3.5 Preparation of Calcium bis {(3R, 5S, 6E)-7-[2-cyclopropyl-4-(4-phenyl)-quinolin-3-yl]-3, 5-dihydroxyhept-6-enoate} (Desfluoro impurity of Pitavastatin calcium)

A suspension of (S)-(-)-α-methyl benzyl amine salt of [(3R, 5S, 6E)-7-[2-cyclopropyl-4-(phenyl)-3-quinolyl]-3, 5-dihydroxy-6-heptenoic acid] (8.0 g) in ethyl acetate (100 ml) and water (100 ml) was cooled to 10^{o}C. pH was adjusted to 3.0 with ~ 35 % aqueous hydrochloric acid solution, stirred and the layers are separated. The organic layer is washed with brine solution, dried and concentrated to get residue. To the residue ethyl acetate and water was added and cooled to 10^{o}C. pH 9.0- 11.0 was adjusted with ~ 4 % aqueous sodium hydroxide solution stirred and separated the layers. Washed the aqueous layer with ethyl acetate. To the aqueous layer was added solution of calcium acetate (1.0 g) in water (50 ml). The suspension was stirred for 3h at room temperature. Filtered and washed with water, dried to give 5.0 of Calcium bis {(3R, 5S, 6E)-7-[2-cyclopropyl-4-(4-phenyl)-quinolin-3-yl]-3, 5-dihydroxyhept-6-enoate}(Desfluoro impurity of Pitavastatin Calcium). HPLC – 99.29 % other impurities - 0.28 %, 0.19 %, Mass m/z = 404 (M + H), 402 (M – H), UV (λ nm) 244, 209, IR (cm-1) 3379 (O-H stretching), 3062, 3027, 3006 (Aromatic C-H stretching), 2938, 2913 (Alipahtic C-H stretching), 1564 (C=OO- asymmetrical stretching), 1485 (Aromatic C=C/C=N stretching), 1442 (Aliphatic C-H bending), 1411 (C=OO- symmetrical stretching), 1215 (C-F stretching), 1117, 1066, 1027 (C-O stretching), 853, 764, 703 (Aromatic C-H bending)

Table 5: NMR data s- singlet, d- doublet, dd- doublet of doublet, t- triplet, m- multiplet, br- broad ^{1}H-^{1}H Coupling constants ^{13}C-^{19}F Coupling constants

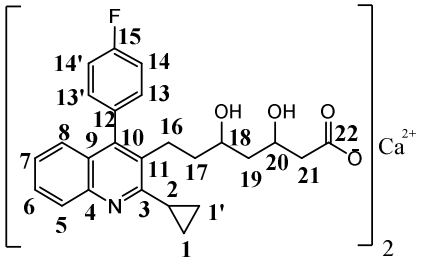


Position	1H	δ (ppm)	J (Hz)[1]	^{13}C, J(Hz)[2]	DEPT
1Ha, 1'Ha	2H	1.01 -1.09	m	10.64, 10.77	CH_2
1Hb, 1'Hb	2H	1.21-1.23	m		
2	1H	2.50	br	15.34	CH
3	-	-	-	160.55	-
4	-	-	-	145.89	-
5	1H	7.84	d (8.4)	125.85	CH
6	1H	7.61	t(7.5)	128.31	CH
7	1H	7.35	t (7.5)	126.17	CH
8	1H	7.42-7.51	m	123.06	CH
9	-	-	-	129.29	-
10	-	-	-	144.63	-
11	-	-	-	125.61	-
12	-	-	-	136.83	-
13, 13'	2H	7.21-7.26	m	128.22, 128.31	CH
14, 14'	2H	7.42-7.51	m	129.64, 129.96	CH
15	1H	7.21-7.26	m	128.73	-
16	1H	6.50	d(16.2)	142.11	CH
17	1H	5.56-5.63	dd (16.2, 5.7)	125.47	CH
18	1H	4.10-4.12	m	68.74	-
19Ha	1H	1.01-1.09	m	43.73	CH_2
19Hb	1H	1.32-1.42	m		
20	1H	3.51-3.52	m	65.46	CH
21Ha	1H	1.81-1.89	dd (14.9, 8.3)	44.52	CH_2
21Hb	1H	1.98-2.05	dd (15.2, 3.2)		
22	-	-	-	177.97	-
OH'	1H	4.88	br	-	-
OH''	**1H**	**6.33**	**br**	-	-

2.3.6 Preparation of Calcium bis {(3R, 5S)-7-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-3,5-dihydroxy heptanoate} (hydrogenated impurity of Pitavastatin calcium)

To a solution of Pitavastatin Calcium (20.0 g) in methanol (200 ml), 10 % Palladium on charcoal (2.0 g) was added and hydrogen gas was passed through the reaction mixture. The reaction mixture was filtered through hyflow bed, and washed with methanol. The filtrate was concentrated under reduced pressure. To the concentrated mass water (200 ml) was added and was cooled to 10^{o}C. pH was adjusted to 9.0- 11.0 with ~ 4 % aqueous sodium hydroxide solution stirred, then added solution of calcium acetate (8.0 g) in water (50 ml). Stirred the suspension for 3 h at room temperature. Filtered and washed with water, dried to give 13.0g of Calcium bis {(3R,5S)-7-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-3,5-dihydroxyheptanoate}(hydrogenated impurity of Pitavastatin calcium), HPLC-88.63 %, Other impurities- 10.64 %, 0.14 %, Mass m/z = 424 (M + H), 422 (M – H), UV (λ nm) 237, 202, IR (cm-1) 3429 (O-H stretching), 2945 (Aliphatic C-H stretching), 1604, 1563 (C=OO- asymmetric stretching), 1513, 1493 (Aromatic C=C/C=N stretching), 1437 (Aliphatic C-H bending), 1413 (C=OO- symmetric stretching), 1222 (C-F stretching), 1158, 1093, 1061, 1026 (C-O stretching), 841, 763 (Aromatic C-H bending)

Table 6: NMR data s- singlet, d- doublet, dd- doublet of doublet, t- triplet, m- multiplet, br- broad ^{1}H-^{1}H Coupling constants ^{13}C-^{19}F Coupling constants



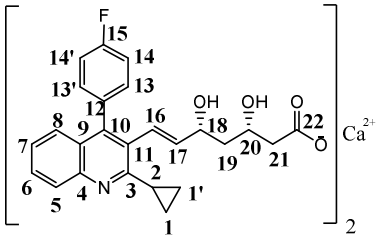
Position	1H	δ (ppm)	J (Hz)[1]	^{13}C, J(Hz)[2]	DEPT
1Ha, 1'Ha	2H	1.01 -1.06	m	10.43, 10.52	CH_2
1Hb, 1'Hb	2H	1.19-1.25	m		
2	1H	2.47-2.50	br	13.99	CH
3	-	-	-	161.49	-
4	-	-	-	145.52	-
5	1H	7.82	d (8.4)	125.30	CH
6	1H	7.55-7.62	t(7.5)	128.23	CH
7	1H	7.31-7.41	t (7.5)	128.23	CH
8	1H	7.08	m	125.47	CH
9	-	-	-	126.04	-
10	-	-	-	144.34	-
11	-	-	-	132.09	-
12	-	-	-	133.13	-
13, 13'	2H	7.31-7.41	m	131.18, d(7.4)	CH
14, 14'	2H	7.31-7.41	m	115.44, d(21.4)	CH
15	-	-	m	161.68, d(242.4)	-
16Ha	1H	2.55-2.64	d(16.2)	25.58	CH_2
16Hb	1H	2.76-2.81			
17	2H	1.51-1.61	dd (16.2, 5.7)	38.10	CH_2
18	1H	3.52	m	66.59	CH
19Ha	1H	1.31-1.41	m	43.32	CH_2
19Hb	1H	1.51-1.61	m		
20	1H	3.77-3.78	m	68.37	CH
21Ha	1H	1.84-1.95	dd (14.9, 8.3)	43.95	CH_2
21Hb	1H	2.04-2.10	dd (15.2, 3.2)		
22	-	-	-	178.56	-
OH'	1H	4.66	br	-	-
OH''	1H	6.08	br	-	-

2.3.7 Preparation of Calcium bis {(3S, 5R, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolin-3-yl]-3, 5-dihydroxy hept-6-enoate} (3S, 5R-Isomer impurity of Pitavastatin Calcium) [(12,)]

A solution of 2-cyclopropyl-4-(4-fluorophenyl)-quinolyl-3-carboxaldehyde (25.0 g), Methyl (3S)-3-(tert-butyldimethylsilyloxy)-5-oxo-6-triphenylphosphoranylidene hexanoate (60.0 g) was refluxed in acetonitrile (100 ml) under heating for 20 h and evaporated under reduced pressure to distill off acetonitrile. The resulting residue was taken into cyclohexane and stirred for 2 h at 15^{o}C. Filtered the reaction mass to remove triphenylphosphine oxide. The filtrate was concentrated under reduced pressure to get thick residue. The residue was taken into acetonitrile (87 ml) was cooled to 0^{o}C, a solution of 82.5 ml ~ 40 % aqueous HF in acetonitrile (87 ml) was added dropwise at 0^{o}C, and the mixture was warmed to room temperature and stirred for 1.5h. To the reaction mixture was added dichloromethane (500 ml) and washed the reaction mass thrice with DM water, then washed with saturated sodium bicarbonate solution and brine solution. The organic layer was dried and concentrated to give Methyl (E)-7-[2-cyclopropyl-4-(4-flurophenyl)-3-quinolinyl]-(3S)-3-hydroxy-5-oxo-6-heptenoate (25.0 g). To 500 ml anhydrous tetrahydrofuran was added 2.5 g sodium borohydride at -78 ^{o}C. To this mixture was added a solution of 57 ml of 1 M diethylmethoxyborane-THF at -78 ^{o}C, and then stirred at same temperature for 30 minutes. To this mixture was slowly added 25 g of Methyl (E)-7-[2-cyclopropyl-4-(4-flurophenyl)-3-quinolinyl]-(3S)-3-hydroxy-5-oxo-6-heptenoate dissolved in 500 ml of anhydrous THF and 175 ml of methanol and the mixture was stirred for 3 h. After conforming the reaction by TLC 31ml acetic acid is added, and the mixture is adjusted to pH 8 with saturated sodium bicarbonate and extracted with ethyl acetate (250 ml). The organic layer is washed with water, dried and the ethyl acetate is evaporated under reduced pressure. To the resulting residue methanol is added and the mixture was evaporated under reduced pressure to give 23 g of (3S, 5R, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3, 5-dihydroxy-6-heptenoic acid. To a solution of (3S, 5R, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3, 5-dihydroxy-6-heptenoic acid (23.0 g) in ethanol (150 ml) was added solution of sodium hydroxide (2.0 g , 0.05 mol) under ice cooling. The reaction mixture was warmed to room temperature and stirred for 2 h. The solvent is distilled

off under reduced pressure. Water (125 ml) and ethyl acetate (250 ml) was added to the resulting residue and was cooled to 10 ^{o}C. pH was adjusted to 3.0 with ~ 35 % aqueous hydrochloric acid solution was stirred and the layers were separated. The organic layer was washed with brine solution, dried and concentrated to get residue. To the residue added methyl tert-butyl ether and water and was cooled to 10 ^{o}C. pH 9.0-11.0 was adjusted with ~ 4 % aqueous sodium hydroxide solution and stirred and the layers were separated. Washed the aqueous layer with Methyl tert-butyl ether. Degassed the aqueous layer under vacuum to remove the traces of MTBE. To the aqueous layer was added solution of Calcium acetate (8.0 g, 0.05 mol) in water (125 ml). Stirred the suspension for 3 h at room temperature. Filtered and washed with water, dried to give Calcium bis {(3S, 5R, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolin-3-yl]-3, 5-dihydroxyhept-6-enoate} (3S, 5R-Isomer impurity of Pitavastatin Calcium) (12.0 g), HPLC- 87.65 % % other impurities – 11.37 %, 0.59 %, MASS m/z = 422 (M + H), 448 (M + Na), 420 (M – H), UV (λ nm) 244, 204, IR (cm-1) 3390- (O-H stretching), 3065, 3007 (Aromatic C-H stretching), 2937 (Aliphatic C-H stretching), 1604 (C=OO- asymmetric stretching), 1513, 1563 (Aromatic C=C/C=N stretching), 1489, 1438 (Aliphatic C-H bending), 1412 (C=OO- symmetric stretching), 1222 (C-F stretching), 1159, 1120, 1066, 1026 (C-O stretching), 843, 763, 724 (Aromatic C-H bending)

Table 7: NMR data s- singlet, d- doublet, dd- doublet of doublet, t- triplet, m- multiplet, br- broad ^{11}H-^{1}H Coupling constants $^{2\,13}$C-^{19}F Coupling constants



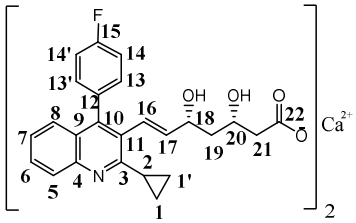
Position	1H	δ (ppm)	J (Hz)[1]	^{13}C, J(Hz)[2]	DEPT
1Ha, 1'Ha	2H	1.01 -1.07	m	10.57, 10.67	CH_2
1Hb, 1'Hb	2H	1.20	br		
2	1H	2.43-2.49	m	15.29	CH
3	-	-	-	160.48	-
4	-	-	-	142.12	-
5	1H	7.85	d (8.1)	128.29	CH
6	1H	7.52-7.68	m	131.36	CH
7	1H	7.24-7.43	m	128.76	CH
8	1H	7.24-7.43	m	123.05	CH
9	-	-	-	129.56	-
10	-	-	-	143.57	-
11	-	-	-	125.56	-
12	-	-	-	133.00, d(3.3)	-
13	2H	7.24-7.43	m	132.03, d(7.9)	CH
13'		7.24-7.43	m	131.75, d(8.2)	CH
14	2H	7.24-7.43	m	115.18, d(21.3)	CH
14'		7.24-7.43	m	115.12, d(21.2)	CH
15	-	-	-	161.53, d(242.9)	-
16	1H	6.48	d(15.6)	145.86	CH_2
17	2H	5.56-5.63	dd (16.2, 5.7)	131.49	CH_2
18	1H	4.12-4.14	m	68.81	CH
19Ha	1H	1.01- 1.07	m	44.18	CH_2
19Hb	1H	1.36-1.45	m		
20	1H	3.56-3.62	m	65.64	CH
21Ha	1H	1.84-1.92	dd (15.3, 8.1)	43.80	CH_2
21Hb	1H	2.02-2.08	dd (15.2, 3.8)		
22	-	-	-	178.52	-
OH'	1H	4.92	br, s	-	-
OH''	1H	6.21	br, s	-	-

2.3.7 Preparation of Calcium bis {(3R, 5R, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolin-3-yl]-3, 5-dihydroxy hept-6-enoate} (3R, 5R-isomer impurity of Pitavastatin Calcium)[(12)]

To 500 ml anhydrous tetrahydrofuran was added 2.5 g sodium borohydride at -78 ^{o}C, and the mixture was stirred at same temperature for 30 minutes. To this mixture was slowly added 25 g Methyl (E)-7-[2-cyclopropyl-4-(4-flurophenyl)-3-quinolinyl]-(3R)-3-hydroxy-5-oxo-6-heptenoate dissolved in 500 ml of anhydrous THF and 175 ml of methanol and the mixture was stirred for 3 hours. After conforming the reaction by TLC 31 ml acetic acid is added thereto, and the mixture is adjusted to pH 8 with saturated sodium bicarbonate and extracted with ethyl acetate (250 ml). The organic layer is washed with water, dried and the ethyl acetate is evaporated under reduced

pressure. To the resulting residue methanol is added and the mixture is evaporated under reduced pressure for three times to give 22.0 g of Methyl [(3R, 5R, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3, 5-dihydroxy-6-heptenoate]. To a solution of Methyl [(3R, 5R, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3, 5-dihydroxy-6-heptenoate] (22.0 g) in ethanol (150 ml) is added solution of sodium hydroxide (2.0 g, 0.05 mol) under ice cooling. The reaction mixture is warmed to room temperature and stirred for 2 h. The solvent is distilled off under reduced pressure. Water (125 ml)and ethyl acetate (250 ml) is added to the resulting residue and was cooled to 10^{o}C. Adjusted pH 3.0 with ~ 35 % Aq.hydrochloric acid solution stirred and separated the layers. The organic layer is washed with brine solution, dried and concentrated to get residue. To the residuewas added methyl tert-butyl ether, water and was cooled to 10^{o}C. pH was adjusted to 9.0-11.0 with ~ 4 % aqueous sodium hydroxide solution, stirred and the layers are separated. The aqueous layer was treated with Methyl tert-butyl ether. Aqueous layer was kept under vacuum to remove the traces of MTBE. To the aqueous layer was added a solution of Calcium acetate (12.0 g) in water (125 ml). The suspension was stirred for 3 h at room temperature. The reaction mass was filtered, washed with water and dried to give Calcium bis {(3R, 5R, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolin-3-yl]-3, 5-dihydroxyhept-6-enoate} (3R, 5R-isomer impurity of Pitavastatin Calcium) (12.0 g), HPLC- 66.80 % Other impurities- 25.08 %, 4.57 %, MASS m/z = 422 (M + H), 448 (M + Na), 420 (M – H), UV (λ nm) 244, 204, IR (cm-1) 3390- (O-H stretching), 3065, 3007 (Aromatic C-H stretching), 2937 (Aliphatic C-H stretching), 1604 (C=OO- asymmetric stretching), 1513, 1563 (Aromatic C=C/C=N stretching), 1489, 1438 (Aliphatic C-H bending), 1412 (C=OO- symmetric stretching), 1222 (C-F stretching), 1159, 1120, 1066, 1026 (C-O stretching), 843, 763, 724 (Aromatic C-H bending)

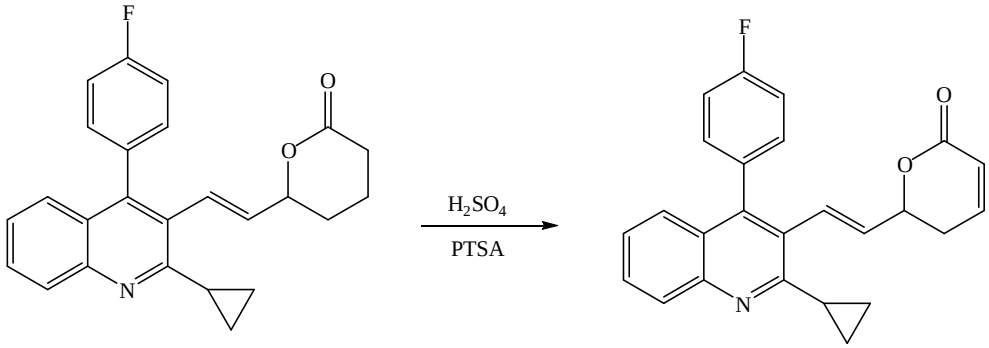
Table 8: NMR data s- singlet, d- doublet, dd- doublet of doublet, t- triplet, m- multiplet, br- broad ^{11}H-^{1}H Coupling constants ^{213}C-^{19}F Coupling constants



Position	1H	δ (ppm)	J (Hz)[1]	^{13}C, J(Hz)[2]	DEPT
1Ha, 1'Ha	2H	1.01 -1.07	m	10.57, 10.67	CH_2
1Hb, 1'Hb	2H	1.20	br		
2	1H	2.43-2.49	m	15.29	CH
3	-	-	-	160.48	-
4	-	-	-	142.12	-
5	1H	7.85	d (8.1)	128.29	CH
6	1H	7.52-7.68	m	131.36	CH
7	1H	7.24-7.43	m	128.76	CH
8	1H	7.24-7.43	m	123.05	CH
9	-	-	-	129.56	-
10	-	-	-	143.57	-
11	-	-	-	125.56	-
12	-	-	-	133.00, d(3.3)	-
13	2H	7.24-7.43	m	132.03, d(7.9)	CH
13'		7.24-7.43	m	131.75, d(8.2)	CH
14	2H	7.24-7.43	m	115.18, d(21.3)	CH
14'		7.24-7.43	m	115.12, d(21.2)	CH
15	-	-	-	161.53, d(242.9)	-
16	1H	6.48	d(15.6)	145.86	CH_2
17	2H	5.56-5.63	dd (16.2, 5.7)	131.49	CH_2
18	1H	4.12-4.14	m	68.81	CH
19Ha	1H	1.02- 1.07	m	44.18	CH_2
19Hb	1H	1.36-1.45	m		
20	1H	3.56-3.62	m	65.64	CH
21Ha	1H	1.84-1.92	dd (15.3, 8.1)	43.80	CH_2
21Hb	1H	2.02-2.08	dd (15.2, 3.8)		
22	-	-	-	178.52	-
OH'	1H	4.92	br, s	-	-
OH''	1H	6.21	br, s	-	-

*2.3.8 Preparation of Dehydrated lactone impurity of Pitavastatin Calcium***:**

Reaction Scheme:

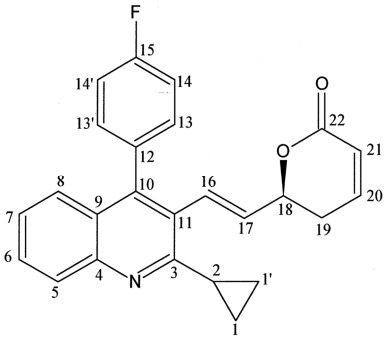


Pitavastatin Lactone impurity **Dehydrated lactone impurity**

A solution of (4R, 6S, E)-6-[2-[2-Cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl] vinyl] tetrahydro-4-hydroxypyran-2-one (Pitavastatin lactone) (10.0 g 0.024 mole), sulfuric acid (2.42 g 0.024) and PTSA (0.02 g) is refluxed in toluene (400 ml) for 4 hours. Then cool the reaction to room temperature. Dilute the reaction mass with dichloromethane (400 ml) and methanol (100 ml) and add water (100ml).Separate the organic layer and with water two times. Dry the organic layer with anhydrous sodium sulphate, distill out the solvent under vacuum, the residue was purified by column chromatography in MDC/Methanol. The product was eluted in 5-10% methanol-MDC mixture , concentrated the product fraction and dried to give (6S)-6-{(E)-2-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]ethenyl}-5,6-dihydro-2H-pyran-2-one MASS m/z 386 (M+H) IR (cm $^{-1}$) 3015 (Aromatic C-H Streching) , 2296 (Aliphatic C-H stretching),1726 (C=O stretching),1629,1603,1571,1512,1489 (Aromatic C=C/C=N stretching), 1401,1390(Aliphatic C-H bending), 1244 ,1218,1161 (C-F stretching), 1070,1057,1018(C-O stretching),846,831,816,774 (Aromatic C-H bending); UV spectra (λ nm) ~ 244

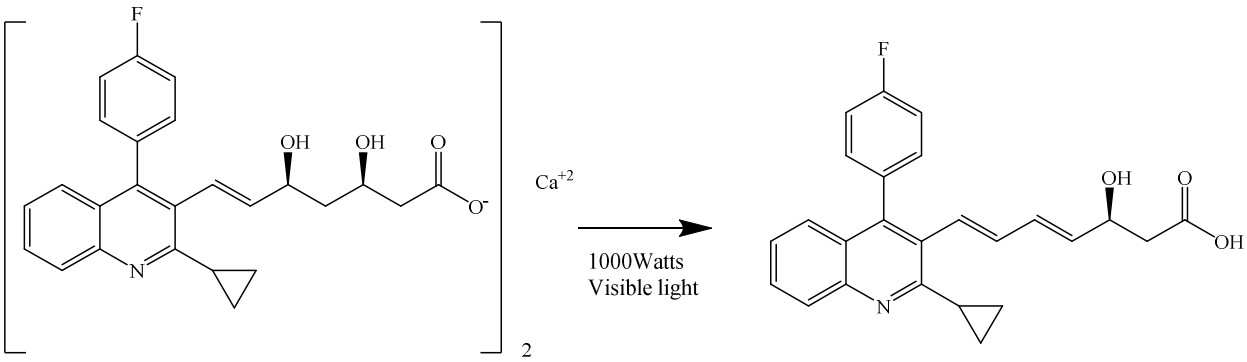
NMR (DMSO – d_6, 300 MHz)

Table 9: NMR data s- singlet, d- doublet, dd- doublet of doublet, t- triplet, m- multiplet, br- broad
^{11}H-^{1}H Coupling constants
^{213}C-^{19}F Coupling constants



Position	1H	δ (ppm)	J (Hz)[1]	^{13}C, J(Hz)[2]	DEPT
1Ha, 1'Ha	2H	1.05 -1.07	m	10.66, 10.69	CH_2
1Hb, 1'Hb	2H	1.21-1.24	m		
2	1H	2.40-2.43	m	15.41	CH
3	-	-	-	160.19	-
4	-	-	-	144.24	-
5	1H	7.88	d (8.0)	125.79	CH
6	1H	7.65-7.69	m	129.18	CH
7	1H	7.29-7.43	m	128.40	CH
8	1H	7.29-7.43	m	125.79	CH
9	-	-	-	128.65	-
10	-	-	-	144.24	-
11	-	-	-	125.45	-
12	-	-	-	132.70	-
13,13'	2H	7.29-7.43	m	132.09, d(7.5) 131.86, d(7.5)	CH
14,14'	2H	7.29-7.43	m	115.29, d(23.8)	CH
15	-	-	-	161.42, d (243.8)	-
16	1H	6.70-6.74	d(16.3,1.3)	135.11	CH
17	1H	5.69-5.73	dd (16.5, 6.0)	127.61	CH
18	1H	5.01-5.05	m	76.84	CH
19Ha	1H	2.09-2.15	m	28.69	CH_2
19Hb	1H	2.31-2.43	m		
20	1H	6.94-6.98	m	146.19	CH
21	1H	5.90-5.93	m	120.28	CH
22	-	-	-	163.07	-

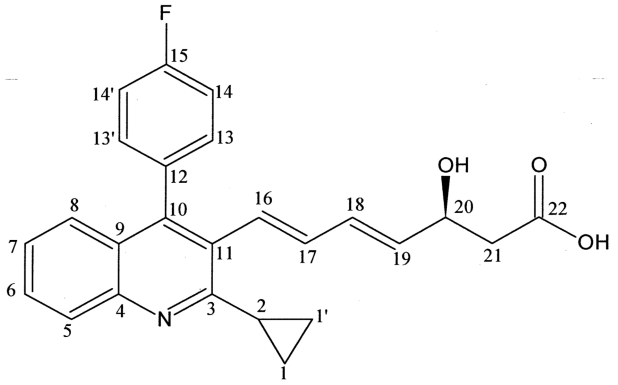
2.3.9 Preparation of 4, 6-diene impurity:
Reaction scheme:



Pitavastatin Calcium **4,6-Diene impurity**

A compound of (3*R*, 5S, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl]-3, 5-dihydroxyhept-6-enoic acid calcium salt[(7)] was taken in petty dish and directly exposed to 1000 Watts visible light for period of 20-24 hrs. Then the crude sample was purified by column chromatography in MDC/Methanol. The product was eluted in 5-10% methanol-MDC mixture concentrated the product fraction and dried to give (3S,4E,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-3-hydroxyhepta-4,6-dienoic acid MASS m/z 404 (M+H) , m/z 402 (M-H), IR (cm^{-1}) 3416,3153 (O-H stretching) ,2296,2855 (Aliphatic C-H stretching),1703 (C=O stretching),1635,1605,1513,1490 (Aromatic C=C/C=N stretching), 1400(Aliphatic C-H bending), 1226 (C-F stretching),1160,1097,1063,1025(C-O stretching),844,765 (Aromatic C-H bending); UV spectra (λ nm) ~ 244

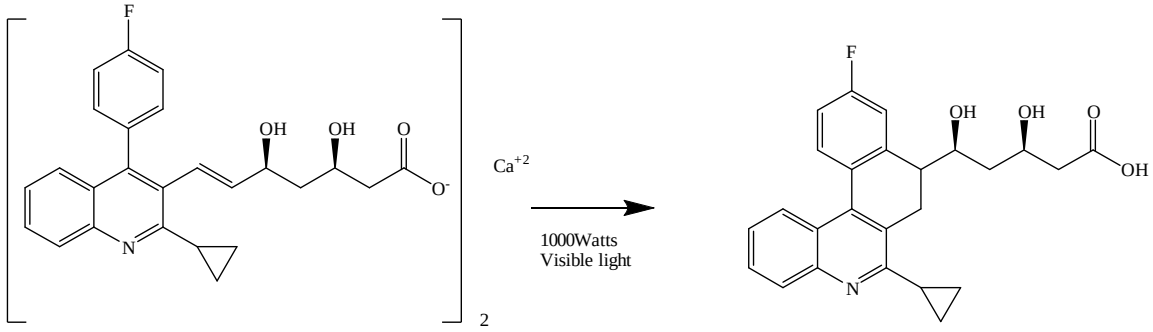
Table 10: NMR data s- singlet, d- doublet, dd- doublet of doublet, t- triplet, m- multiplet, br- broad
^{11}H-^{1}H Coupling constants
^{213}C-^{19}F Coupling constants



Position	1H	δ (ppm)	J (Hz)[1]	^{13}C, J(Hz)[2]	DEPT
1Ha, 1'Ha	2H	1.00 -1.04	m	10.74	CH_2
1Hb, 1'Hb	2H	1.18-1.23	m		
2	1H	1.18-1.23	m	15.40	CH
3	-	-	-	160.41	-
4	-	-	-	143.69	-
5	1H	7.86	d (8.1)	125.65	CH
6	1H	7.61-7.67	m	128.86	CH
7	1H	7.24-7.42	m	128.34	CH
8	1H	7.24-7.42	m	125.62	CH
9	-	-	-	129.30	-
10	-	-	-	145.93	-
11	-	-	-	125.52	-
12	-	-	-	132.92, d(2.5)	-
13,13'	2H	7.24-7.42	m	131.86, d(7.5) 131.97, d(7.5)	CH
14,14'	2H	7.29-7.43	m	115.24, d(21.3)	CH
15	-	-	-	161.60, d (243.8)	-
16	1H	6.49-6.54	d(16.2,1.2)	145.22	CH
17	1H	5.61-5.69	dd (16.1, 5.7)	124.07	CH
18	1H	6.66-6.76	m	141.09	CH
19	1H	5.73	d(15.6)	123.64	CH
20	1H	4.12-4.16	m	69.52	CH
21	2H	2.18	t(6.3)	39.16	CH_2
22	-	-	-	166.93	-
23	1H	5.11	d(4.8)	-	-

2.3.10 Preparation of Aromatic alkene impurity[(13)]:

Reaction scheme:

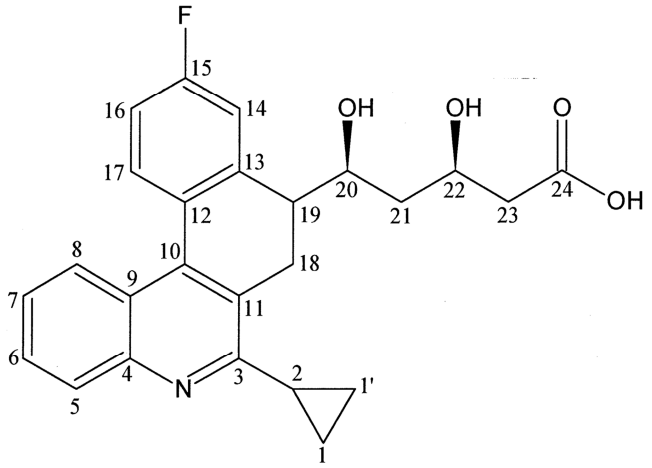


Pitavastatin Calcium **Aromatic alkene impurity**

A solution of (3*R*, 5S, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl]-3, 5-dihydroxyhept-6-enoic acid calcium salt (10.0 g 0.011 mole) in Acetonitrile (950 ml) and water (550 ml) and the reaction mass exposed to 1000 watts visible light for 22-24 hrs. Then distill out the solvent under vacuum, dilute the reaction mass with ethyl acetate (200ml) and water (100 ml) and adjust the reaction mass pH-2.0-4.0 by using (1:4) HCl solution. Separate the layers and wash the organic layer with water and followed by 10% brine solution. Dry the organic layer with anhydrous sodium sulfate, distill out the solvent under vacuum. The obtained residue was purified using Prep.HPLC to give (3R,5S)-5-(6-cyclopropyl-10-fluoro-7,8-dihydrobenzo[k] phenanthridine-8-yl)-3,5-dihydroxypentanoic acid MASS m/z 422 (M+H) , m/z 420 (M-H), IR (cm $^{-1}$) 3393 (O-H stretching) ,3010 (Aromatic C-H stretching),2918 (Aliphatic C-H stretching),1715 (C=O stretching),1635,1607,1593,1573,1489 (Aromatic C=C/C=N stretching), 1400(Aliphatic C-H bending), 1345 (OH bending),1318,1276,1237,1184,1159 (C-F stretching),1097,1060 (C-O stretching),873,831,774,762 (Aromatic C-H bending); UV spectra (λ nm) ~ 320 and ~ 225

NMR (DMSO – d_6, 300 MHz)

Table 11: NMR data s- singlet, d- doublet, dd- doublet of doublet, t- triplet, m- multiplet, br- broad
^{11}H-^{1}H Coupling constants
^{213}C-^{19}F Coupling constants



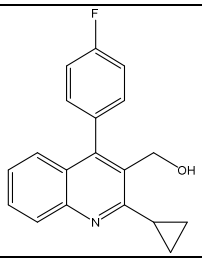
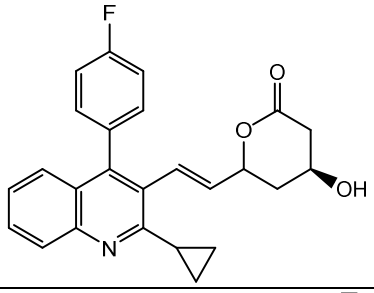
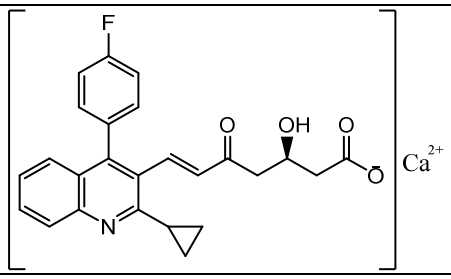
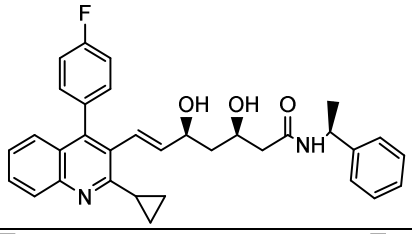
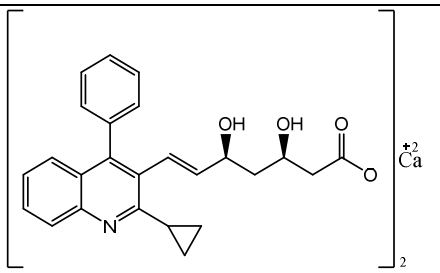
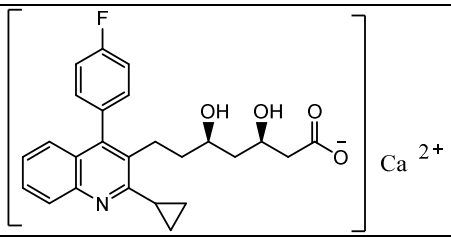
Position	1H	δ (ppm)	J (Hz)[1]	^{13}C, J(Hz)[2]	DEPT
1, 1’	4H	0.96-1.34	m	8.78,9.53	CH_2
2	1H	2.44	m	14.16	CH
3	-	-	-	160.43	-
4	-	-	-	147.04	-
5	1H	8.25-8.30	m	125.50	CH
6	1H	7.65	t(12.8)	129.03	CH
7	1H	7.48-7.53	m	124.74	CH
8	1H	7.87-7.95	m	128.07	CH
9	-	-	-	122.18	-
10	-	-	-	137.13	-
11	-	-	-	127.74	-
12	-	-	-	131.12	-
13	-	-	-	143.59, d(7.5)	-
14	1H	7.24-7.41	m	116.12, d(22.5)	CH
15	-	-	-	161.69, d(246.3)	-
16	1H	7.24-7.41	m	113.39, d(21.3)	CH
17	1H	7.87-7.95	m	130.86, d(7.5)	CH
18	2H	5.01-5.05	m	24.87	CH_2
19	1H	3.38	br	43.96	CH
20	1H	3.63-3.68	m	67.02	CH
21	2H	1.55-1.69	m	41.65	CH_2
22	1H	3.86-3.97	m	65.99	CH
23	2H	1.94-2.18	m	42.15	CH_2
24	-	-	-	172.99	-

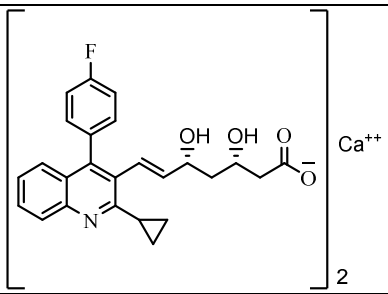
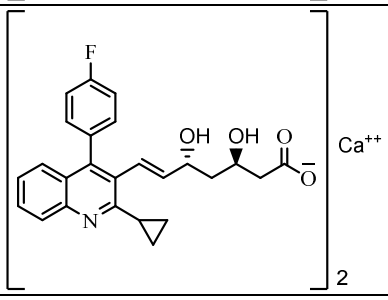
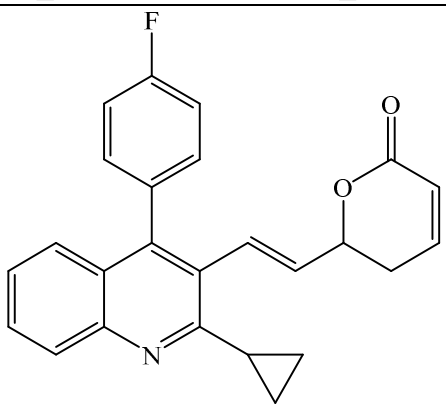
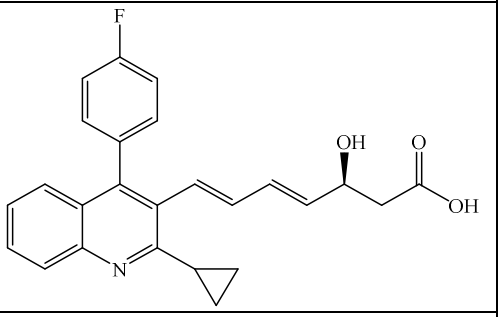
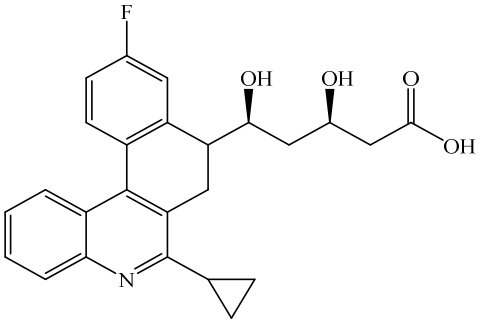
RESULTS AND DISCUSSION

In the literature, many processes are described for the synthesis of Pitavastatin calcium [4-17]. An important route for the synthesis of Pitavastatin is in which 4-(4-flourophenyl)-6-isopropyl-2-(N-methyl-N-methylsulfonyl amino)-

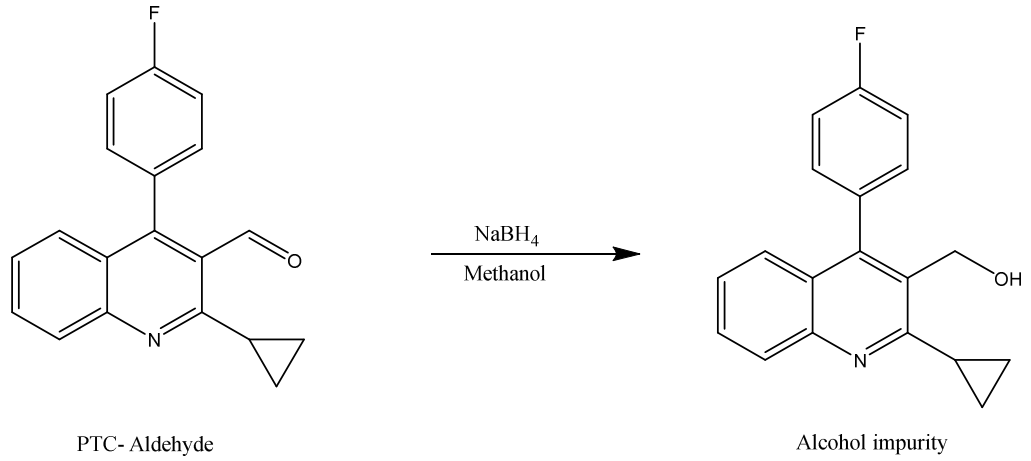
5-pyrimidine carbaldehyde is reacted with Methyl (3R)-3-(tert-butyldimethylsilyloxy)-5-oxo-6-triphenylphosphoranylidene hexanoate, then cleavage of OTBDMS group with hydrofluoric acid, then selective reduction of keto group by using diethyl methoxyborane and sodium borohydride , then reaction with sodium hydroxide to cleave methyl ester then with calcium source (calcium chloride Or Calcium acetate) to get Pitavastatin calcium. Literature also reports several impurities related to Pitavastatin calcium [18]. There are several process related impurities which are observed and identified (See Figure 1).

Table 12: Structures of the impurities

S. No.	Name of the impurity	Structure
01	Alcohol impurity	
02	Lactone impurity	
03	5-Keto acid impurity	
04	Amide impurity	
05	Desfluoro impurity	
06	Hydrogenated impurity	

07	3S,5R-Isomer impurity	
08	3R, 5R-isomer impurity	
09	Dehydrated lactone impurity	
10	4, 6-diene impurity	
11	**Aromatic alkene impurity**	

3.1 Pathway for Alcohol impurity of Pitavastatin Calcium

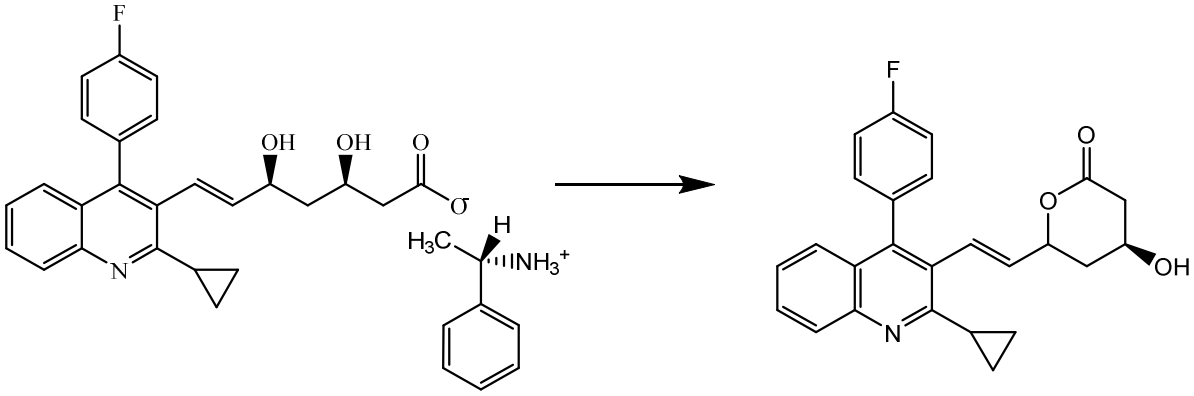


PTC- Aldehyde Alcohol impurity

Scheme 1. Preparation of of [2-cyclopropyl-4(4-fluorophenyl)quinolin-3-yl]methanol impurity (Alcohol impurity of Pitavastatin)

Alcohol impurity was prepared by reduction of PTC aldehyde (A pitavastatin intermediate)

3.2 Pathway for lactone impurity of Pitavastatin Calcium



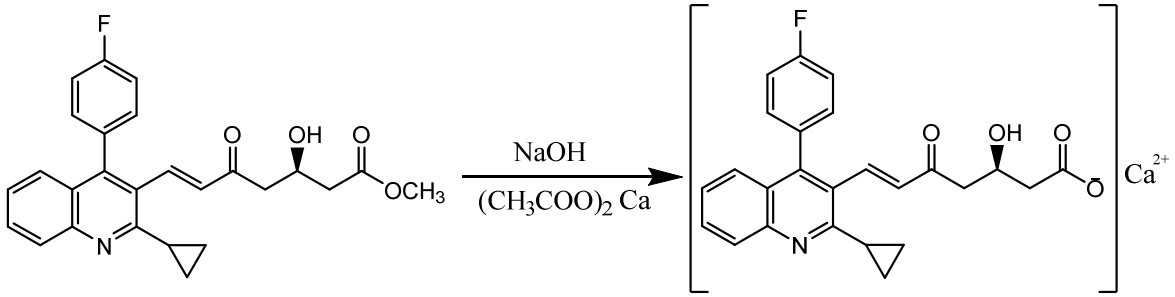
(S)-(-) alpha-methyl benzyl amine salt of [(3R, 5S, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3, 5-dihydroxy-6-heptenoic acid]

Lactone impurity

Scheme 2: Synthetic route for preparation of Lactone impurity of Pitavastatin calcium

(S)-(-)-α-methyl benzyl amine salt of [(3R, 5S, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3, 5-dihydroxy-6-heptenoic acid] is acidified to pH 3 using hydrochloric acid solution and then heated in toluene to give Lactone impurity.

3.3 Pathway for 5- keto acid impurity of Pitavastatin Calcium



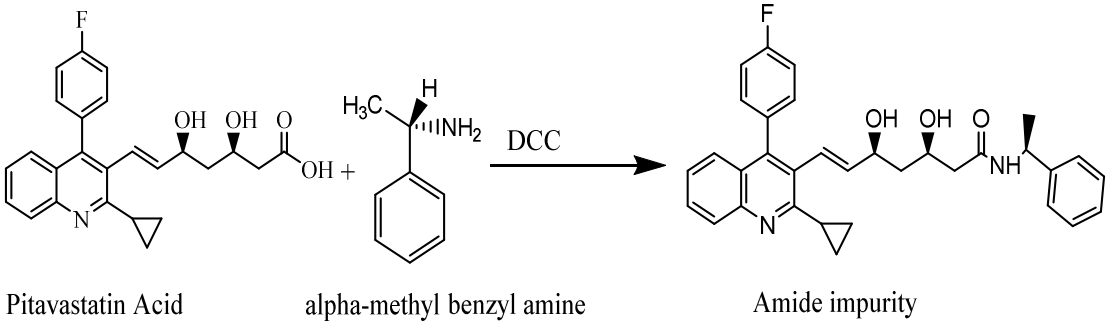
Methyl (E)-7-[2-cyclopropyl-4-(4-flurophenyl)-3-quinolinyl]- (3R)-3-hydroxy-5-oxo-6-heptenoate

5- keto acid impurity

Scheme 3: Synthetic route for preparation of 5-keto acid impurity of Pitavastatin Calcium

Methyl (E)-7-[2-cyclopropyl-4-(4-flurophenyl)-3-quinolinyl]-(3R)-3-hydroxy-5-oxo-6-heptenoate is treated with sodium hydroxide and calcium acetate to give 5- keto acid impurity

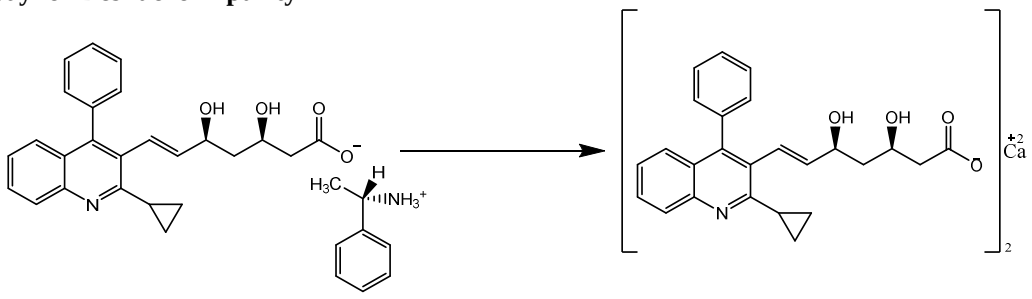
3.4 Pathway for Amide impurity



Scheme 4: Synthetic route for preparation of Amide impurity of Pitavastatin calcium

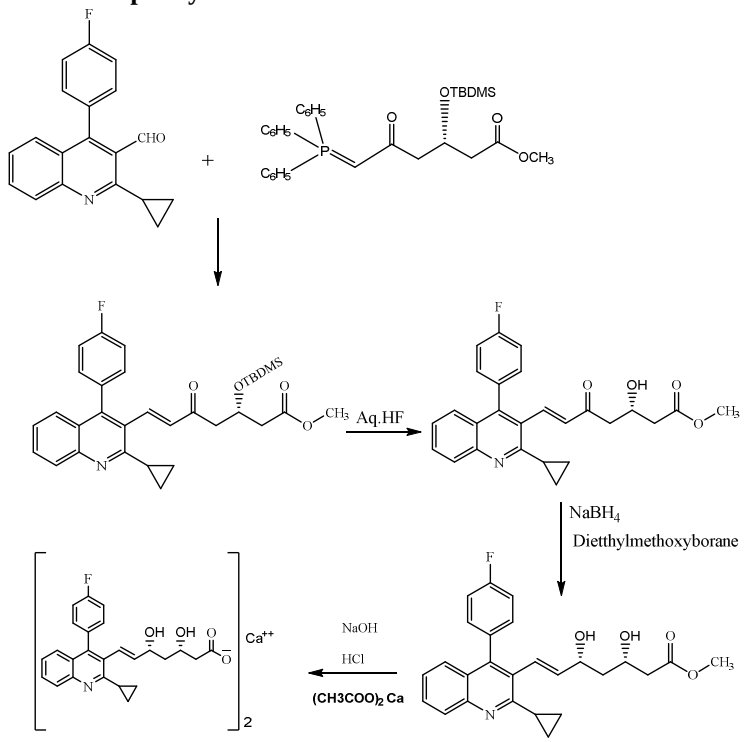
Amide impurity is prepared by condensation of pitavastatin acid and α-methyl benzyl amine in DCC.

3.5 Pathway for Desfluoro impurity



(S)-(-)- alpha-methyl benzyl amine salt of
[(3R, 5S, 6E)-7-[2-cyclopropyl-4-(phenyl)-3-quinolyl]-
3, 5-dihydroxy-6-heptenoic acid]

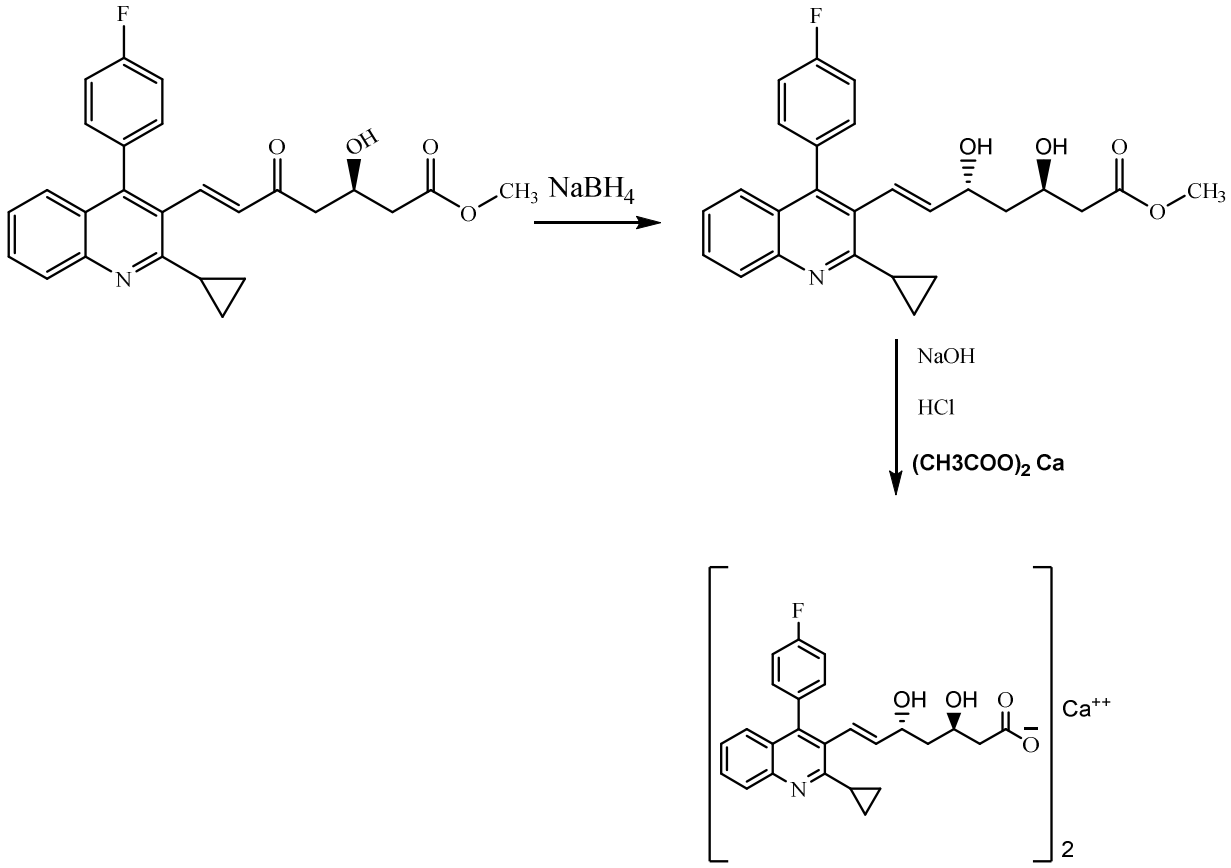
3.6 Pathway for 3S, 5R isomer impurity



Scheme 7: Synthetic route for preparation of 3S, 5R-Isomer impurity of Pitavastatin Calcium

For preparation of 3S, 5R-Isomer impurity 2-cyclopropyl-4-(4-fluorophenyl)-quinolyl-3-carboxaldehyde is reacted with Methyl (3S)-3-(tert-butyldimethylsilyloxy)-5-oxo-6-triphenylphosphoranylidene hexanoate in presence of triphenylphsophine to give Methyl (E)-7-[2-cyclopropyl-4-(4-flurophenyl)-3-quinolinyl]-(3S)-3-hydroxy-5-oxo-6-heptenoate which further reacts with diethylmethoxyborane-THF to give (3S, 5R, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3, 5-dihydroxy-6-heptenoic acid. The compound obtained was basified by using sodium hydroxide and treated with calcium acetate to give (3S, 5R-Isomer impurity of Pitavastatin Calcium).

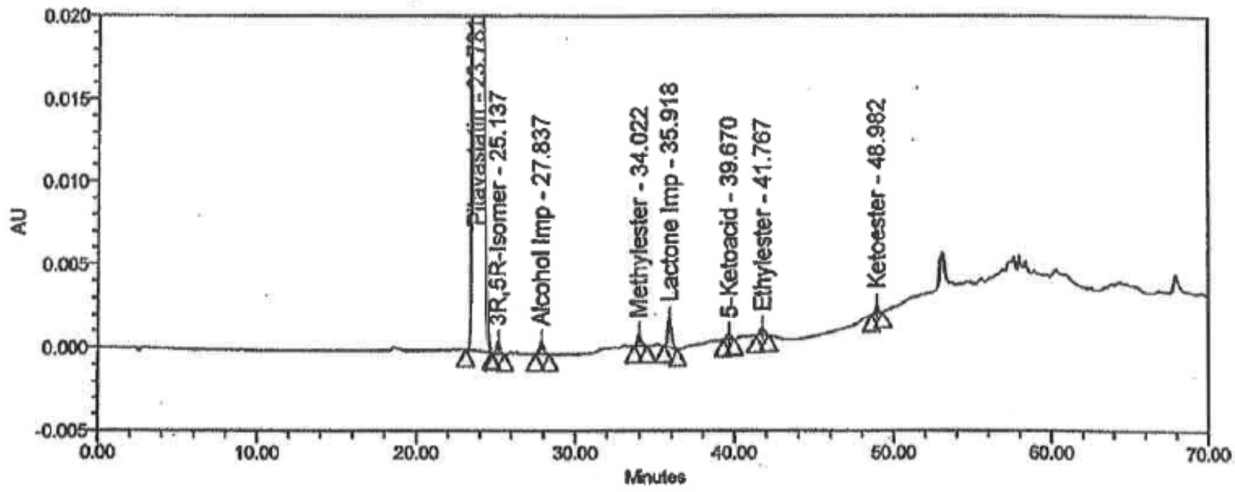
3.7 Pathway for 3R, 5R isomer impurity



Scheme 8: Synthetic route for preparation of 3R, 5R isomer impurity of Pitavastatin Calcium

The preparation of 3R, 5R isomer involves treatment of Methyl (E)-7-[2-cyclopropyl-4-(4-flurophenyl)-3-quinolinyl]-(3R)-3-hydroxy-5-oxo-6-heptenoate with sodiumborohydride to give Methyl [(3R, 5R, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3, 5-dihydroxy-6-heptenoate]. The compound thus obtained is treated with sodium hydroxide and finally treated with calcium acetate to get 3R, 5R isomer impurity of Pitavastatin.

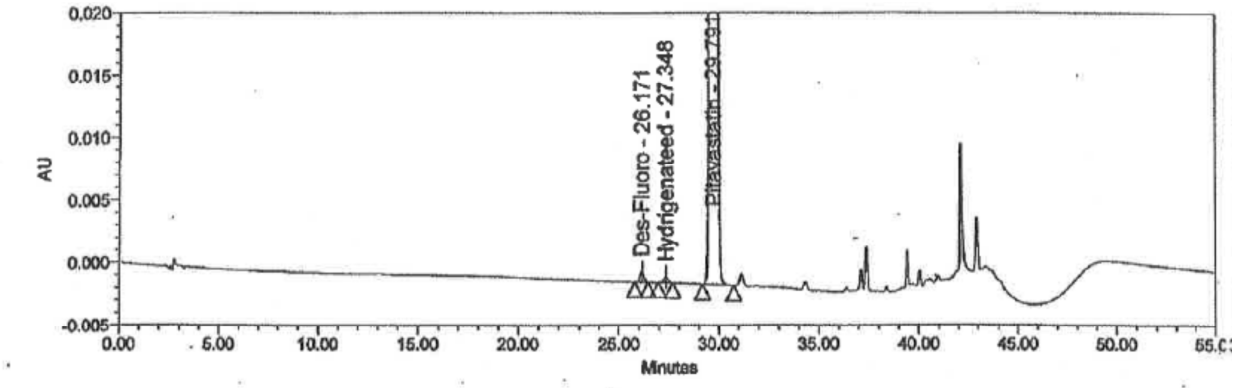
All the compounds prepared was characterized by MASS, NMR, IR, UV and HPLC. A typical chromatogram of Pitavastatin with its impurities has been given below (Figure 2 and 3). A separate HPLC chiral method was used for determining the chiral impurities of Pitavastatin like 3S, 5R and 3R, 3R isomers (Figure 4).



Peak Results

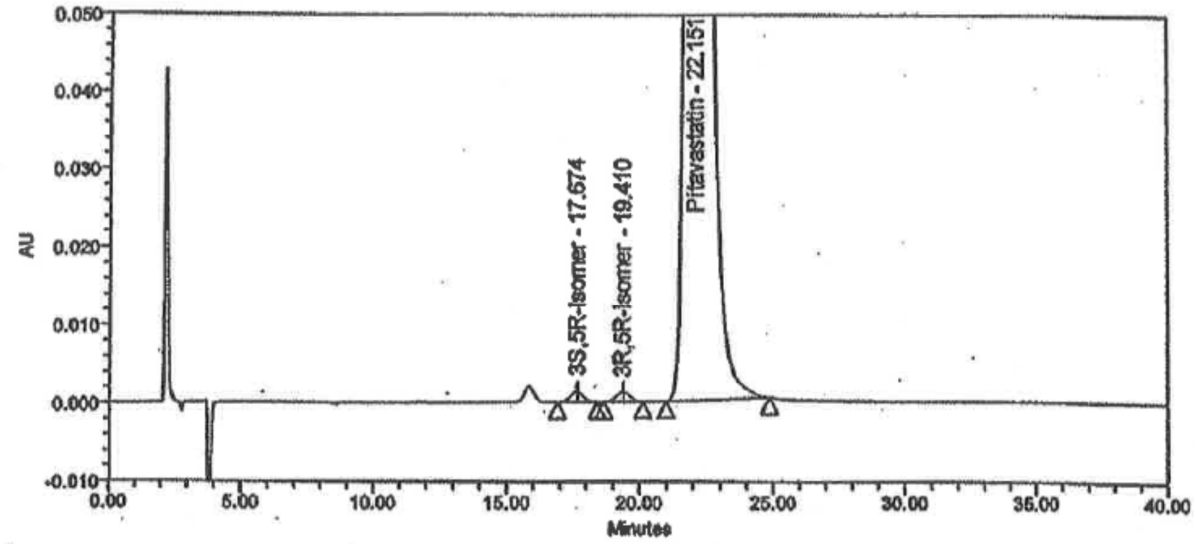
	Name	RT	Area	% Area	RT Ratio	USP Resolution	USP Plate Count	USP Tailing
1	Pitavastatin	23.781	11814909	99.28			44990	1.31
2	3R,5R-Isomer	25.137	11453	0.10	1.06	2.93	51126	1.34
3	Alcohol Imp	27.837	12151	0.10	1.17	5.79	61043	1.32
4	Methylester	34.022	11529	0.10	1.43	13.77	108833	1.32
5	Lactone Imp	35.918	28996	0.24	1.51	4.32	109742	1.33
6	5-Ketoacid	39.670	5712	0.05	1.67	7.48	79868	1.03
7	Ethylester	41.767	8912	0.07	1.76	3.83	98481	1.08
8	Ketoester	48.982	6922	0.06	2.06	15.42	267188	0.94

Figure 1. HPLC of Pitavastatin with related substances



	Name	RT	Area	% Area	RT Ratio	USP Resolution	USP Plate Count	USP Tailing
1	Des-Fluoro	26.17	11263	0.09	0.88		81688.47	0.95
2	Hydrigenateed	27.35	6900	0.06	0.92	3.13	84887.86	1.00
3	Pitavastatin	29.79	12011908	99.85	1.00	6.71	122171.46	0.96
Sum			12030070.6					

Figure 2. HPLC of Pitavastatin with related substances



Peak Results

	Name	RT	Area	% Area	RT Ratio	Resolution	USP Tailing	USP Plate Count
1	3S,5R-isomer	17.674	45748	0.28	0.80		1.01	5835.13
2	3R,5R-isomer	19.410	45526	0.28	0.88	1.85	1.02	6370.17
3	Pitavastatin	22.151	15961996	99.43		2.61	1.24	5942.21

Figure 3. Chiral HPLC of Pitavastatin with its chiral isomers

CONCLUSION

Impurities related to pitavastatin has been synthesized and characterized by NMR, MASS, IR, UV. During preparation of Pitavastatin calcium, impurities in the range of 0.03 to 0.15 % were observed. These impurities were identified by their mass numbers in LC-MS and were then prepared and isolated. Identification of these impurities could help in improvement of yield and quality of pitavastatin prepared.

Acknowledgement
Our group thanks Department of Scientific and Industrial Research India, Dr. Hari Babu (CEO Mylan Laboratories Ltd India), Mr. Sanjeev Sethi (Head Mylan Global R & D), Dr Yasir Rawjee {Head - Global API (Active Pharmaceutical Ingredients)}, Dr. Ramesh Dandala (Head MLL R & D), Dr. Suryanarayana Mulukutla (Head Analytical Dept MLL R & D) as well as analytical development team of Mylan Laboratories Limited for their encouragement and support. We would also like to thanks Dr Narahari Ambati (Head IPR MLL R & D) & his Intellectual property team for their support

REFERENCES

[1] Ahmed, S., Madsen, C.S., Stein, P.D. *Journal of Medicinal Chemistry* 51 (**2008**) 2722-2733
[2] Baumann, K.L. *Tetrahedron Letters* 33(19**9**2) 2283-2284
[3] Casar, Z., Steinbocher, M., Kosmrlj. *Journal of Organic Chemistry* 75(**2010**) 6681-6684
[4]Gotto, A. M.; Smith, L. C. "IXth International Symposium Drugs Affecting Lipid Metabolism" (**1986**) 30-31.
[5] Johnson, D.S.; Li, J.J. Art of Drug Synthesis, Wiley, New York(**2007**) 169-182.
[6]Miyachi, N., Yanagawa, Y., Iwasaki, H. *Tetrahedron Letters* 34(**1993**) 8267-8270
[7]Reddy, M. S.; Eswaraiah, S.; Reddy, M. S. IN **2009CH00120** A 20100730
[8]Stone, M.T. *Organic Letters* 13 (**2011**) 2326-.2329
[**9**]Turner, K. *Organic Process Research and Development* 8(**2004**) 823-.833
[10]Castorer, J., Sorbera, L.A., Leeson, P.A.NK-104. Hypolipidemic, HMG-CoA reductase inhibitor Drugs of the Future 23(**1998**) 847-.
[11]Endo, A. *Journal of Medicinal Chemistry* 28(**1985**) 401-405
[12]Evans, D. A., Hoveyda, A. H. *Journal of Organic chemistry* 55(**1990**) 5190-5192
[13] Grobelny, P., Viola, G, Vedaldi, D., et al., *Journal of Pharm. And Biomedical Analysis*, 50 (**2009**) 597-601