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Synthesis and characterization of process-related impurities of an anti-hyperuricemia drug-Febuxostat

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ABSTRACT

Febuxostat is a urate-lowering drug, indicated for the treatment of hyperuricemia and gout. During the process development of febuxostat, nine process related impurities were observed at a level of 0.1-0.15 area percent. Synthesis and characterization of these impurities and investigation of their formation is described.

Keywords: Febuxostat; impurites; synthesis; characterization, gout

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INTRODUCTION

Febuxostat is a xanthine oxidase inhibitor used in the treatment of gout[1-3] caused by excessive levels of uric acid in the blood (hyperuricemia)[4]. The uric acid forms crystals in joints (gouty arthritis) and tissues, causing inflammation and pain. Elevated blood uric acid levels also can cause kidney disease and kidney stones. Febuxostat prevents the production of uric acid by blocking the activity of the enzyme (xanthine oxidase) that converts purines to uric acid.

Impurity removal is a critical and important task in pharmaceutical process reseach, where in the final product is required to meet stringent purity requirements. The presence of impurities in an active pharmaceutical ingredient (API) can have significant impact on the quality and safety of the drug product. International Conference on Harmonization (ICH) of technical requirment for registration of pharmaceuticals for human use recommends identifying and characterising all impurities present in APIs at a level of 0.10 % and more. Standards of these impurities are required in pure form to study the impurity profile of API and also for the development of an accurate analytical method[5-6] during the research and development phase.

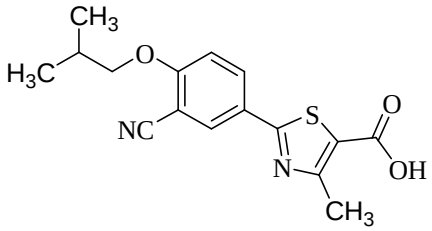


Fig. 1. Structure of Febuxostat 1

During the course of development of an improved and scalable process for Febuxostat in our laboratories, nine process related impurities **5-13** were observed in reaction mass at different stages at a level of 0.1-5.0 area percent.

Herein, we report the complete impuritiy profile of febuxostat including indentification and synthesis of these related substances **5-13.**

MATERIALS AND METHODS

2.1. Measurements

The ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ and DMSO-d_6 using 300 MHz, on a Bruker AVANCE-II 300 MHz FT NMR spectrometer; the chemical shifts (δ_H, δ_C) are reported in δ ppm relative to TMS. The FT-IR spectra (υ_{max} in cm^{-1}) were recorded in the solid state as KBr dispersion using Perkin-Elmer 1650 FT-IR spectrophotometer. The mass spectrum (70eV) was recorded on HP-5989A LC–MS spectrometer. The purity of compounds was checked by HPLC (Shimadzu LC Solution). Reaction monitoring was done by TLC (0.5 mm thickness) using silica gel-G coated Al-plates (Merck), mobile phase: Ethylacetate: Hexane (6:4) and spots were visualized by exposing the dry plates in UV light (254.0 nm) or iodine vapours. The melting points were determined by using the capillary method on POLMON (model MP-96) melting point apparatus and are uncorrected. The elemental analysis (C, H, N) of compounds was performed on Carlo Erba-1108 elemental analyzer. Their results were found to be in good agreement with the calculated values. The solvents and reagents were used without further purification.

2.2. Synthesis

*2.2.1. Synthesis of ethyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (**5**)* [7-8]

A solution of 3-cyano-4-isobutoxybenzothioamide (**2**) (5.0 g, 0.021 mol.) and ethyl-2-chloro-3-oxobutanoate (**3**) (4.42 mL, 0.032 mol.) in isopropanol (25.0 mL) at 25-30°C was heated 3-4 h at 75-80°C. The mixture was allowed to cool up to 25-30°C and the precipitated pale yellowish solid was filtrated, washed with isopropanol (10.0 mL) and dried into vacuum tray drier at 50-55°C under vacuum to give ethyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (**5**).

Yield: 94.0 % (6.90 g), mp 240°C. Purity by HPLC: 99.65 % (23.91 min. retention time), Anal. Calcd for $C_{18}H_{20}N_2O_3S$: C, 62.77; H, 5.85; N, 8.13. Found: C, 62.81; H, 5.83; N, 8.31 %; IR (KBr) υ_{max} (in cm^{-1}): 3394.83, 3070.78, 2974.33, 2874.03, 2804.59, 2588.56, 2225.93, 1894.16, 1820.86, 1708.99, 1604.83, 1512.24, 1469.81, 1427.37, 1396.51, 1369.50, 1300.07, 1261.49, 1168.90, 1099.46, 1041.60, 1014.59, 956.72, 910.43, 825.56, 756.12, 725.26, 655.82, 636.53, 613.38, 551.66, 493.79; ^{1}H NMR (300 MHz, $CDCl_3$ or DMSO-d_6) δ_H (in ppm): 1.09-1.11 (d, 6H, $(C\underline{H}_3)_2$>CH-), 2.15-2.28 (m, 1H, $(CH_3)_2$>C$\underline{H}$-), 3.90-3.92 (d, 2H, >CH-C$\underline{H}_2$−), 7.01–8.19 (d, dd, 3H, Ar-$\underline{H}$), 2.78 (s, 3H, -C$\underline{H}_3$), 4.33-4.40 (q, 2H, -OC$\underline{H}_2$-CH$_3$), 1.38-1.42 (t, 3H, -OCH$_2$-C$\underline{H}_3$); ^{13}C NMR (300 MHz, DMSO–d_6) δ_C (in ppm) (Position[a]): 167.0 (l), 162.3 (p), 161.9 (n), 160.9 (e), 132.4 (g), 131.9 (i), 125.9 (m), 121.8 (h), 115.3 (k), 112.5 (f), 102.8 (j), 75.5 (d), 61.3 (q), 28.0 (b), 18.9 (a, c), 17.3 (o), 14.2 (r); MS *m/z* (%) (70 eV): *m/z* =345.0 (100.0 %) [M+1], 346.0 (17.0 %) [M+2], 347.0 (5.0 %) [M+3].

[a] Refer structure formula of compound **5** in **Fig. 2** for numbering.

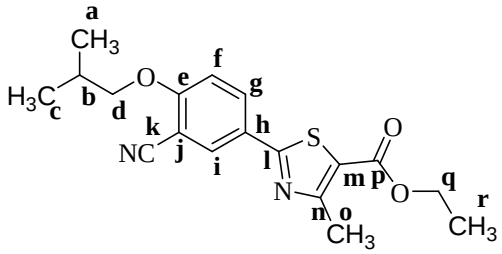


Fig. 2. Ethyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (5), [*]Numbering has been assigned for ^{13}C NMR characterization

*2.2.2 Synthesis of 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (**1 tech grade**)*[9]

To a stirred solution of ethyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (**5,** 5.0 g, 0.014 mol.) in n-butanol (50.0 mL) at 25-30°C, was added NaOH (1.74 g, 0.043 mol.) and reaction mass heated for 1-2 h at 35-40°C. The reaction mixture was allowed to cool up to 25-30°C, and pH was adjusted to 1-2 using con. HCl (5.0 mL) at 25-30°C. Precipitated product was filtrated, washed with n-butanol: water (1:1) (10.0 mL) and dried in vacuum tray drier at 50-55°C under vacuum to provide febuxostat (**1 tech grade)**. Yield: 87.0 % (4.0 g), mp 239°C. Purity by HPLC: 99.40 % (10.2 min. retention time).

*2.2.3. Synthesis of 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (**1**)*[10]

A solution of 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (**1 tech grade,** 5.0 g, 0.015 mol.) in methanol (50.0 mL) was heated the reaction mass at 60-65°C till clear solution was obtained. Water (50.0 mL) was added drop wise into reaction mass with in 30.0 min. at 60-65°C. Resultant white crystalline solid was filtrated,

washed with water (10.0 mL) and dried in vacuum tray drier at 50-55°C under vacuum to give 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (**1**).

Yield: 95.0 % (4.75 g) mp 239°C. Purity by HPLC: 99.74 % (10.2 min. retention time), Anal. Calcd for $C_{16}H_{16}N_2O_3S$: C, 60.74; H, 5.10; N, 8.85. Found: C, 60.70; H, 5.11; N, 8.87 %; IR (KBr) υ_{max} (in cm^{-1}): 3834.61, 3742.03, 3680.30, 3556.85, 3456.55, 2962.76, 2877.89, 2661.85, 2546.12, 2353.23, 2229.79, 2168.06, 2029.18, 1921.16, 1790.00, 1674.27, 1604.83, 1512.24, 1427.37, 1381.08, 1280.78, 1172.76, 1118.75, 1010.73, 918.15, 833.28, 771.55, 725.26, 648.10, 524.66, 462.93; ^{1}H NMR (300 MHz, $CDCl_3$ or DMSO-d_6) δ_H (in ppm): 1.00-1.02 (d, 6H, $(C\underline{H}_3)_2$-CH-), 2.49-2.50 (m, 1H, $(CH_3)_2$-C$\underline{H}$-), 3.97-3.99 (d, 2H, -CH-C$\underline{H}_2$−), 7.33–8.25 (d, dd, 3H, Ar-$\underline{H}$), 2.64 (s, 3H, -C$\underline{H}_3$), 13.39 (s, 1H, -COO$\underline{H}$); ^{13}C NMR (300 MHz, DMSO–d_6) δ_C (in ppm) (Position[a]): 166.3 (l), 162.9 (p), 162.2 (n), 159.6 (e), 133.1 (g), 131.6 (i), 125.5 (m), 123.0 (h), 115.5 (k), 114.0 (f), 101.7 (j), 75.2 (d), 27.7 (b), 18.8 (a, c), 17.1 (o); MS *m/z* (%) (70 eV): *m/z* =317.0 (100.0 %) [M+1], 318.0 (16.0 %) [M+2], 403.0 (63.0 %), 512.0 (47.0 %), 482.0 (46.0 %), 405.0 (27.0 %), 468.0 (25.0 %), 570.0 (24.0 %).

[a] Refer structure formula of compound (**1**) in **Fig. 4** for numbering.

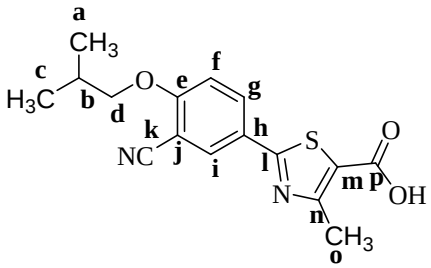
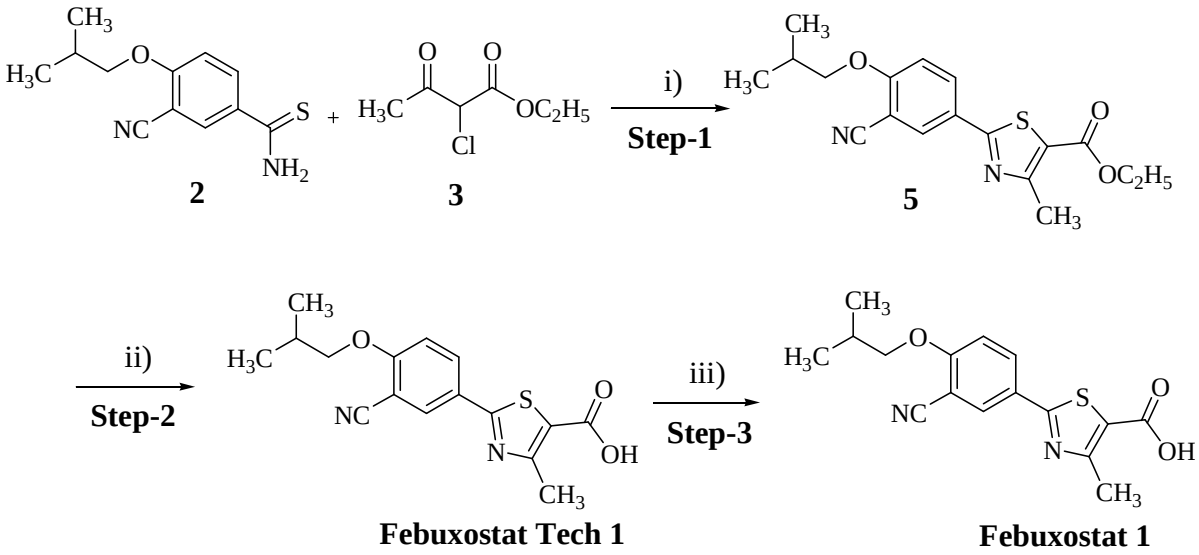


Fig. 4. 2-(3-Cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (1),
[*]Numbering has been assigned for ^{13}C NMR characterization



Scheme 1. Synthesis of febuxostat 1: (i) Thioamide 2, halo diketo compound 3, isopropyl alcohol, reflux (75-80°C); (ii) NaOH, n-butanol, 35-40°C; (iii) Methanol, water, reflux (60-65°C)

*2.2.4. General procedure for substituted alkyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (febuxostat ester impurities) (**4-7**)*

A solution of 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (**1**) (5.0 g, 0.016 mol.) and con. H_2SO_4 (3.0-4.0 mL) in alcoholic solvent (50.0 mL) was stirred at 25-30°C and was heated for 10-12 h at reflux temperature based on boiling point of the alcoholic solvent. The reaction mass was allowed to cool up to 25-30°C and solvent was distilled off at 50-55°C under vacuum to obtain oily residue. Residue was dissolved in methylene dichloride (100.0 mL) at 25-30°C and washed with 5.0 % $NaHCO_3$ solution (3 X 20.0 mL). Methylene dichloride layer was separated, dried over Na_2SO_4 (2.0 g) and distilled at 40-45°C under vacuum to provide compound **5-8** which was recrystallised from acetone (50.0 mL). [Alcoholic solvents like methanol, ethanol, isopropanol and n-butanol were used respectively, to prepare compounds **4**, **5**, **6** and **7**].

*2.2.4.1. Spectral data of febuxostat ester impurities **4-7***

*2.2.4.1.1 Methyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (**4**)*

Yield 82.0 % (4.28 g), mp 225°C. Purity by HPLC: 99.190 % (21.372 min. retention time), Anal. Calcd for $C_{17}H_{18}N_2O_3S$: C, 61.80; H, 5.49; N, 8.48. Found: C, 61.83; H, 5.44; N, 8.43 %; IR (KBr) υ_{max} (in cm^{-1}): 3367.82, 3120.93, 3074.63, 3012.91, 2962.76, 2877.89, 2854.74, 2225.93, 1817.00, 1693.56, 1662.69, 1604.83, 1508.38, 1469.81, 1435.09, 1381.08, 1346.36, 1300.07, 1257.63, 1172.76, 1126.47, 1103.32, 1049.31, 1003.02, 960.58, 914.29, 833.28, 806.27, 759.98, 732.97, 659.68, 613.38, 582.38, 551.66, 493.79, 451.78, 412.78; ^{1}H NMR (300 MHz, $CDCl_3$ or DMSO-d_6) δ_H (in ppm): 1.01-1.03 (d, 6H, $(C\underline{H}_3)_2$>CH-), 2.07-2.20 (m, 1H, $(CH_3)_2$>C<u>H</u>-), 3.82-3.84 (d, 2H, >CH-C<u>H</u>$_2$−), 6.92–8.10 (d, dd, 3H, Ar-<u>H</u>), 2.69 (s, 3H, -C<u>H</u>$_3$), 3.82 (s, 3H, -OC<u>H</u>$_3$); MS *m/z* (%) (70 eV): *m/z* =331.0 (100.0 %) [M+1], 472.0 (21.0 %), 471.0 (14.0 %), 469.0 (10.0 %), 358.0 (15.0 %), 332.0 (12.0 %), 380.0 (6.0 %).

*2.2.4.1.2. Ethyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (**5**)*

Yield 85.0 % (4.63 g), mp 240°C. Purity by HPLC: 99.657 % (23.914 min. retention time), Anal. Calcd for $C_{18}H_{20}N_2O_3S$: C, 62.77; H, 5.85; N, 8.13. Found: C, 62.81; H, 5.83; N, 8.31 %; IR (KBr) υ_{max} (in cm^{-1}): 3394.83, 3070.78, 2974.33, 2874.03, 2804.59, 2588.56, 2225.93, 1894.16, 1820.86, 1708.99, 1604.83, 1512.24, 1469.81, 1427.37, 1396.51, 1369.50, 1300.07, 1261.49, 1168.90, 1099.46, 1041.60, 1014.59, 956.72, 910.43, 825.56, 756.12, 725.26, 655.82, 636.53, 613.38, 551.66, 493.79; ^{1}H NMR (300 MHz, $CDCl_3$ or DMSO-d_6) δ_H (in ppm): 1.09-1.11 (d, 6H, $(C\underline{H}_3)_2$>CH-), 2.15-2.28 (m, 1H, $(CH_3)_2$>C<u>H</u>-), 3.90-3.92 (d, 2H, >CH-C<u>H</u>$_2$−), 7.01–8.19 (d, dd, 3H, Ar-<u>H</u>), 2.78 (s, 3H, -C<u>H</u>$_3$), 4.33-4.40 (q, 2H, -OC<u>H</u>$_2$-CH_3), 1.38-1.42 (t, 3H, -OCH_2-C<u>H</u>$_3$); ^{13}C NMR (300 MHz, DMSO–d_6) δ_C (in ppm) (Position[a]): 167.0 (l), 162.3 (p), 161.9 (n), 160.9 (e), 132.4 (g), 131.9 (i), 125.9 (m), 121.8 (h), 115.3 (k), 112.5 (f), 102.8 (j), 75.5 (d), 61.3 (q), 28.0 (b), 18.9 (a, c), 17.3 (o), 14.2 (r); MS *m/z* (%) (70 eV): *m/z* =345.0 (100.0 %) [M+1], 346.0 (17.0 %) [M+2], 347.0 (5.0 %) [M+3].

[a] Refer structure formula of compound **5** in **Fig. 5** for numbering.

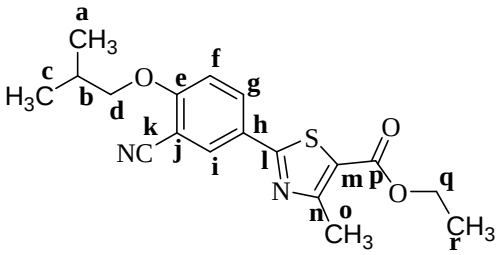


Fig. 5. Ethyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (5), [*]Numbering has been assigned for ^{13}C NMR characterization

[a] Refer structure formula of compound **6** in **Fig. 6** for numbering.

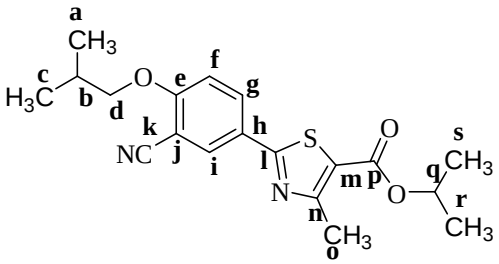


Fig. 6. Isopropyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (7), [*]Numbering has been assigned for ^{13}C NMR characterization

*2.2.4.1.3. Isopropyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (**6**)*

Yield 80.0 % (4.53 g), mp 202°C. Purity by HPLC: 98.436 % (25.688 min. retention time), Anal. Calcd for $C_{19}H_{22}N_2O_3S$: C, 63.66; H, 6.19; N, 7.82. Found: C, 63.67; H, 6.23; N, 7.84 %; IR (KBr) υ_{max} (in cm^{-1}): 3614.72, 3390.97, 3120.93, 3070.78, 2974.33, 2955.04, 2874.03, 2700.43, 2588.56, 2333.94, 2225.93, 2090.91, 2017.61, 1913.45, 1708.99, 1666.55, 1604.83, 1577.82, 1512.24, 1465.95, 1431.23, 1369.50, 1300.07, 1265.35, 1172.76, 1126.47, 1091.75, 1041.60, 1014.59, 960.58, 914.29, 825.56, 759.98, 725.26, 636.53, 613.38, 551.66, 493.79, 439.78; ^{1}H NMR (300 MHz, $CDCl_3$ or DMSO-d_6) δ_H (in ppm): 1.00-1.03 (d, 6H, $(C\underline{H}_3)_2$>CH-), 2.06-2.20 (m, 1H, $(CH_3)_2$>C<u>H</u>-), 3.81-3.83 (d, 2H, >CH-C<u>H</u>$_2$−), 6.92–8.10 (d, dd, 3H, Ar-<u>H</u>), 2.68 (s, 3H, -C<u>H</u>$_3$), 5.09-5.18 (m, 1H, -OC<u>H</u><$(CH_3)_2$), 1.28-1.30 (d, 6H, -OCH<$(C\underline{H}_3)_2$); ^{13}C NMR (300 MHz, DMSO–d_6) δ_C (in ppm) (Position[a]): 166.9 (l), 162.4 (p), 161.6 (n), 160.7 (e), 132.4 (g), 132.0 (i), 126.1 (m), 122.4 (h), 115.3 (k), 112.5 (f), 102.9 (j), 75.6 (d), 69.1

(q), 28.1 (b), 21.9 (s, r), 19.0 (a, c), 17.4 (o); MS *m/z* (%) (70 eV): *m/z* =359.0 (100.0 %) [M+1], 470.0 (12.0 %), 360.0 (10.0 %).

*2.2.4.1.4. Butyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (**7**)*

Yield 88.0 % (5.18 g), mp 158°C. Purity by HPLC: 98.723 % (30.030 min. retention time), Anal. Calcd for $C_{20}H_{24}N_2O_3S$: C, 64.49; H, 6.49; N, 7.52. Found: C, 64.53; H, 6.53; N, 7.50 %; IR (KBr) υ_{max} (in cm^{-1}): 3375.54, 3205.80, 3174.94, 3120.93, 3082.35, 3063.06, 3032.20, 2966.62, 2931.90, 2874.03, 2364.81, 2337.80, 2287.65, 2233.64, 2009.89, 1940.45, 1898.02, 1836.29, 1759.14, 1701.27, 1604.83, 1570.11, 1512.24, 1454.38, 1435.09, 1392.65, 1369.50, 1319.35, 1276.92, 1168.90, 1099.46, 1045.45, 1010.73, 968.30, 918.15, 840.99, 763.84, 732.97, 640.39, 617.24, 551.66, 497.65, 435.93; ^{1}H NMR (300 MHz, $CDCl_3$ or DMSO-d_6) δ_H (in ppm): 1.01-1.03 (d, 6H, $(C\underline{H}_3)_2$>CH-), 2.07-2.29 (m, 1H, $(CH_3)_2$>C$\underline{H}$-), 3.82-3.84 (d, 2H, >CH-C$\underline{H}_2$−), 6.92–8.11 (d, dd, 3H, Ar-$\underline{H}$), 2.69 (s, 3H, -C$\underline{H}_3$), 4.21-4.25 (t, 2H, -OC$\underline{H}_2$-CH_2-CH_2-CH_3), 1.62-1.71 (m, 2H, -OCH_2-C$\underline{H}_2$-CH_2-CH_3), 1.34-1.50 (m, 2H, -OCH_2-CH_2-C$\underline{H}_2$-CH_3), 0.89-0.94 (t, 3H, -OCH_2-CH_2-CH_2-C$\underline{H}_3$); ^{13}C NMR (300 MHz, DMSO–d_6) δ_C (in ppm) (Position[a]): 167.1 (l), 162.4 (p), 162.1 (n), 161.0 (e), 132.5 (g), 132.0 (i), 126.0 (m), 122.0 (h), 115.3 (k), 112.5 (f), 102.9 (j), 75.6 (d), 65.2 (q), 30.6 (r), 28.1 (b), 19.2 (a, c), 19.0 (s), 17.4 (o), 13.7 (t); MS *m/z* (%) (70 eV): *m/z* =373.0 (100.0 %) [M+1], 374.0 (16.0 %) [M+2], 375.0 (3.0 %) [M+3].

[a] Refer structure formula of compound **7** in **Fig. 7** for numbering.

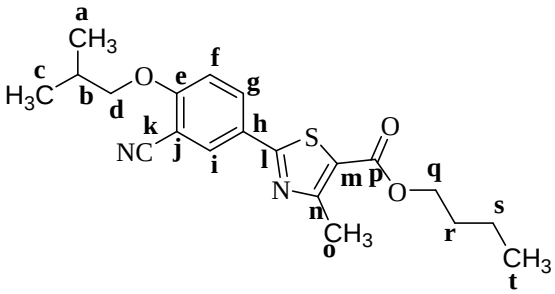
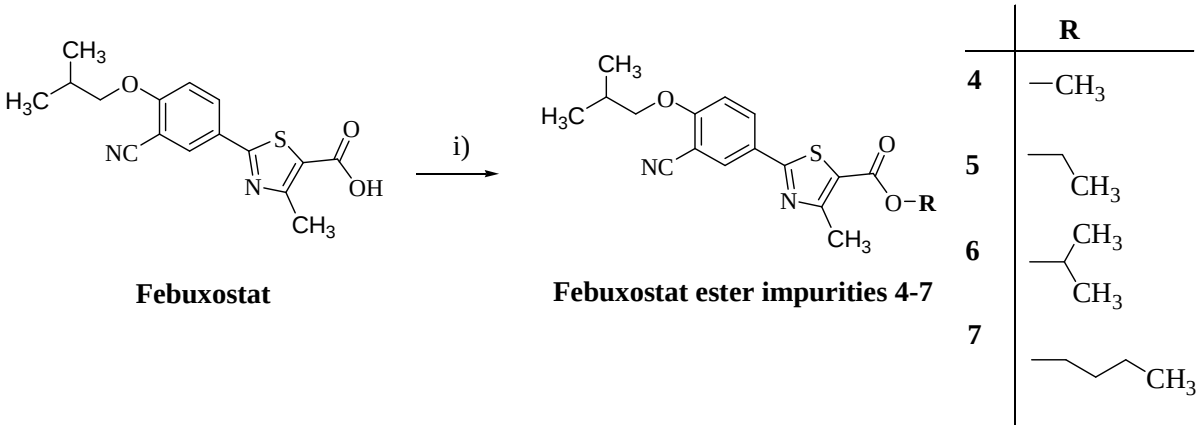


Fig. 7. Butyl-2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate (7), [*]Numbering has been assigned for ^{13}C NMR characterization



Febuxostat **Febuxostat ester impurities 4-7**

Scheme 2 Synthesis of febuxostat ester impurities: (i) Esterification reaction: Con. H_2SO_4, alcoholic solvents [methanol for impurity 4, ethanol for impurity 5, isopropanol for impurity 6 and n-butanol for impurity 7], reflux temperature based on boiling points of repective alcoholic solvents

*2.2.5. Procedure for 2-(3-carbamoyl-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (febuxostat amide impurity) (**8**)*

A solution of 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (**1**) (5.0 g, 0.016 mol.) in dimethylsulfoxide (50.0 mL), cooled in an ice-water bath (0-5°C), are added anhydrous K_2CO_3 powder (5.0 g) and drop wise 30.0 % H_2O_2 (35.0 mL) within 30.0 min. at 0-5°C. The reaction mass was allowed to warm up to 25-30°C (strong exothermic effect is observed), and maintained at 25-30°C for 24.0 hrs under stirring. Water (100.0 mL) was added into reaction mass at 25-30°C, and pH was adjusted to1-2 using con. HCl (15.0 mL) at 25-30°C to obtain white solid which was recrystallised from acetone (50.0 mL) to give febuxostat amide impurity **8**.

Yield: 90.0 % (4.75 g), mp 258°C. Purity by HPLC: 99.530 % (4.356 min. retention time), Anal. Calcd for $C_{16}H_{18}N_2O_4S$: C, 57.47; H, 5.43; N, 8.38. Found: C, 57.50; H, 5.40; N, 8.35 %; IR (KBr) υ_{max} (in cm^{-1}): 3402.54, 3290.67, 3186.51, 3066.92, 2970.48, 2928.04, 2874.03, 2820.02, 2654.14, 2619.42, 2542.26, 2368.66, 2337.80, 2276.08, 2229.79, 2106.34, 2033.04, 1863.30, 1755.28, 1678.13, 1647.26, 1597.11, 1523.82, 1469.81, 1427.37,

1377.22, 1323.21, 1292.35, 1273.06, 1257.63, 1226.77, 1161.19, 1118.75, 1099.46, 1041.60, 1018.45, 925.86, 813.99, 763.84, 721.40, 705.97, 644.25, 613.38, 570.95, 528.51, 466.79, 443.64, 401.21; ^{1}H NMR (300 MHz, $CDCl_3$ or DMSO-d_6) δ_H (in ppm): 0.99-1.01 (d, 6H, $(C\underline{H}_3)_2$>CH-), 2.06-2.19 (m, 1H, $(CH_3)_2$>C$\underline{H}$-), 3.96-3.98 (d, 2H, >CH-C$\underline{H}_2$−), 7.25–8.34 (d, dd, 3H, Ar-$\underline{H}$), 2.66 (s, 3H, -C$\underline{H}_3$), 13.36 (s, 1H, -COO$\underline{H}$), 7.60-7.77 (s, 2H, -CON$\underline{H}_2$); ^{13}C NMR (300 MHz, DMSO–d_6) δ_C (in ppm) (Position[a]): 167.8 (l), 165.8 (p), 163.1 (k), 159.8 (n), 158.8 (e), 130.5 (g), 128.9 (i), 124.9 (m), 124.0 (h), 122.2 (j), 113.9 (f), 75.1 (d), 27.7 (b), 19.2 (a, c), 17.2 (o); MS *m/z* (%) (70 eV): *m/z* =335.0 (100.0 %) [M+1], 336.0 (25.0 %) [M+2], 472.0 (13.0 %), 431.0 (9.0 %), 493.0 (8.0 %).

[a] Refer structure formula of compound **8** in **Fig. 8** for numbering.

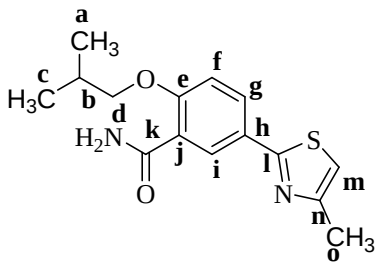


Fig. 8. 2-(3-Carbamoyl-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (febuxostat amide impurity) (8), *Numbering has been assigned for ^{13}C NMR characterization

*2.2.6. Procedure for 2-(3-carboxy-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (febuxostat diacid impurity) (**9**)*

To a stirred solution of 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (**1,** 5.0 g, 0.016 mol.) in n-butanol (85.0 mL) and water (85.0 mL) at 25-30°C, was added NaOH (6.32 g, 0.16 mol.). Reaction mass was heated for 48 h at 100-110°C, and was allowed to cool up to 25-30°C. pH of the reaction mass was adjusted to 1-2 using con. HCl (25.0 mL) at 25-30°C. Resulted white solid was filtrated, washed with n-butanol: water (1:1) (10.0 mL) and dried in vacuum tray drier at 50-55°C under vacuum to give febuxostat diacid impurity **9**.

Yield: 95.0 % (5.03 g), mp 268°C. Purity by HPLC: 99.070 % (5.186 min. retention time), Anal. Calcd for $C_{16}H_{17}NO_5S$: C, 57.30; H, 5.11; N, 4.18. Found: C, 57.33; H, 5.08; N, 4.20 %; IR (KBr) υ_{max} (in cm^{-1}): 3537.57, 3514.42, 3367.82, 3182.65, 3128.64, 3082.35, 3047.63, 2955.04, 2928.04, 2874.03, 2638.71, 2526.83, 2376.38, 2337.80, 1928.88, 1909.59, 1855.58, 1820.86, 1689.70, 1600.97, 1577.82, 1508.38, 1469.81, 1423.51, 1377.22, 1330.93, 1292.35, 1253.77, 1219.05, 1168.90, 1091.75, 1049.31, 1022.31, 956.72, 918.15, 852.56, 825.56, 798.56, 756.12, 732.97, 678.97, 613.38, 574.81, 528.51, 474.50; ^{1}H NMR (300 MHz, $CDCl_3$ or DMSO-d_6) δ_H (in ppm): 0.99-1.01 (d, 6H, $(C\underline{H}_3)_2$>CH-), 1.98-2.11 (m, 1H, $(CH_3)_2$>C$\underline{H}$-), 3.88-3.90 (d, 2H, >CH-C$\underline{H}_2$−), 7.22–8.22 (d, dd, 3H, Ar-$\underline{H}$), 2.66 (s, 3H, -C$\underline{H}_3$), 13.04, 13.28 (s, 2H, -COO$\underline{H}$); ^{13}C NMR (300 MHz, DMSO–d_6) δ_C (in ppm) (Position[a]): 167.7 (k), 166.8 (l), 163.1 (m), 159.9 (n), 159.8 (e), 131.3 (g), 128.9 (i), 124.4 (m), 122.3 (h), 122.1 (f), 114.1 (j), 74.7 (d), 27.9 (b), 19.1 (a, c), 17.2 (o); MS *m/z* (%) (70 eV): *m/z* =336.0 (100.0 %) [M+1], 337.0 (11.0 %) [M+2], 472.0 (8.0 %), 380.0 (6.0 %).

[a] Refer structure formula of compound **9** in **Fig. 9** for numbering.

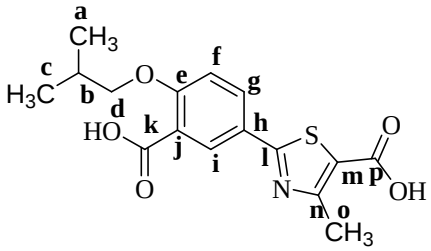
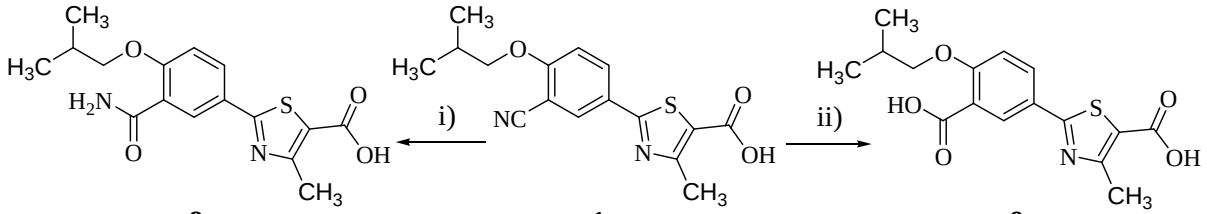


Fig. 9. 2-(3-Carboxy-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (febuxostat diacid impurity) (9), *Numbering has been assigned for ^{13}C NMR characterization

*2.2.7. Procedure for 2-isobutoxy-5-(4-methylthiazol-2-yl)benzonitrile (febuxostat des acid impurity) (**10**)*

To a stirred solution of 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (**1,** 5.0 g, 0.016 mol.) in glacial acetic acid (30.0 mL) was added con. H_2SO_4 (2.5 mL) and water (20.0 mL) and reaction mixture was heated under 100-110°C temperature for 24 h (until no more carbon dioxide is evolved). The reaction mass was chilled in an ice bath, made alkaline with 20.0 % sodium hydroxide solution (130.0 mL) and extracted with toluene (3 X 50.0 mL). The combine toluene layers were washed with water (2 X 50.0 mL) and dried over anhydrous Na_2SO_4 (5.0 g), and solvent was removed by distillation under vacuum at 50-55°C to give febuxostat des acid impurity **10**.



Scheme 3 Synthesis of febuxostat amide 8 and febuxostat diacid 9 impurities: (i) H_2O_2 (30.0 %), DMSO, anhydrous K_2CO_3 powder, 0-5°C; (ii) n-Butanol, NaOH, water, reflux (100-110°C)

Yield 80.0 % (3.44 g), mp 148 °C. Purity by HPLC: 87.936 % (14.568 min. retention time), Anal. Calcd for $C_{15}H_{16}N_2OS$: C, 66.15; H, 5.92; N, 10.29. Found: C, 66.18; H, 5.95; N, 10.33 %; IR (KBr) υ_{max} (in cm^{-1}): 3904.05, 3834.61, 3742.03, 3680.30, 3510.56, 3464.27, 3279.10, 3078.49, 2962.76, 2877.89, 2731.29, 2677.29, 2576.98, 2468.97, 2360.95, 2291.51, 2222.07, 2137.20, 2036.90, 1928.88, 1735.99, 1705.13, 1612.54, 1566.25, 1512.24, 1458.23, 1388.79, 1280.78, 1172.76, 1118.75, 1010.73, 964.44, 918.15, 871.85, 833.28, 779.27, 725.26, 648.10, 540.09, 493.79; ^{1}H NMR (300 MHz, $CDCl_3$ or DMSO-d_6) δ_H (in ppm): 1.00-1.03 (d, 6H, ($C\underline{H}_3$)$_2$>CH-), 2.02-2.15 (m, 1H, (CH_3)$_2$>C$\underline{H}$-), 3.97-3.99 (d, 2H, >CH-C$\underline{H}_2$−), 7.33–8.20 (s, d, dd, 4H, Ar-$\underline{H}$), 2.50 (s, 3H, -C$\underline{H}_3$); ^{13}C NMR (300 MHz, DMSO–d_6) δ_C (in ppm) (Position[a]): 164.9 (l), 162.0 (e), 154.0 (n), 133.3 (g), 131.3 (i), 126.8 (h), 116.3 (k), 115.5 (f), 114.5 (m), 101.9 (j), 75.7 (d), 28.2 (b), 19.3 (a, c), 17.3 (o); MS *m/z* (%) (70 eV): *m/z* =273.0 (100.0 %) [M+1], 274.0 (19.0 %) [M+2], 275.0 (7.0 %) [M+3], 336 (5.0 %).

[a] Refer structure formula of compound **10** in **Fig. 10** for numbering.

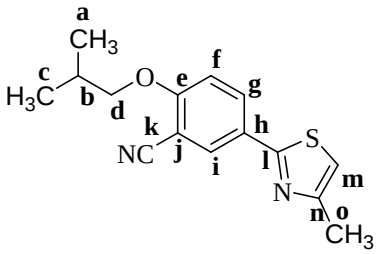


Fig. 10. 2-Isobutoxy-5-(4-methylthiazol-2-yl)benzonitrile (febuxostat des acid impurity) (10), *Numbering has been assigned for ^{13}C NMR characterization

*2.2.8. Procedure for 2-isobutoxy-5-(4-methylthiazol-2-yl)benzamide (febuxostat amide-des acid impurity) (**11**)*
To a stirred solution of 2-(3-carbamoyl-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (**8,** 5.0 g, 0.016 mol.) in glacial acetic acid (30.0 mL) was added con. H_2SO_4 (2.5 mL) and water (30.0 mL) and reaction mixture was heated under 100-110°C temperature for 10-12 h or until no more carbon dioxide is evolved. The reaction mass was chilled in an ice bath, made alkaline with 20.0 % sodium hydroxide solution (120.0 mL) and extracted with toluene (3X50.0 mL). The combine toluene layers were washed with water (2X50.0 mL) and dried over anhydrous Na_2SO_4 (10.0 g) and the solvent was distilled off to give febuxostat amide-des acid impurity **11**.

Yield 78.0 % (3.57 g), mp 158°C. Purity by HPLC: 99.597 % (5.770 min. retention time), Anal. Calcd for $C_{15}H_{18}N_2O_2S$: C, 62.04; H, 6.25; N, 9.65. Found: C, 62.08; H, 6.20; N, 9.62 %; IR (KBr) υ_{max} (in cm^{-1}): 3398.69, 3290.67, 3263.66, 3186.51, 2966.62, 2924.18, 2870.17, 2777.59, 2507.54, 2360.95, 2333.94, 1855.58, 1724.42, 1643.41, 1597.11, 1504.53, 1442.80, 1377.22, 1253.77, 1222.91, 1161.19, 1122.61, 1095.60, 1022.31, 960.58, 933.58, 922.00, 860.28, 810.13, 767.69, 702.11, 644.25, 617.24, 563.23, 520.80, 486.08, 435.93; ^{1}H NMR (300 MHz, $CDCl_3$ or DMSO-d_6) δ_H (in ppm): 1.00-1.02 (d, 6H, ($C\underline{H}_3$)$_2$>CH-), 2.06-2.19 (m, 1H, (CH_3)$_2$>C$\underline{H}$-), 3.95-3.97 (d, 2H, >CH-C$\underline{H}_2$−), 7.22–8.30 (s, d, dd, 4H, Ar-$\underline{H}$), 2.41 (s, 3H, -C$\underline{H}_3$), 7.59-7.74 (s, 2H, -CON$\underline{H}_2$); ^{13}C NMR (300 MHz, DMSO–d_6) δ_C (in ppm) (Position[a]): 165.9 (l), 165.6 (k), 157.9 (e), 153.2 (n), 129.9 (g), 128.5 (i), 125.9 (h), 123.6 (j), 114.1 (m), 113.8 (f), 75.1 (d), 27.7 (b), 19.2 (a, c), 17.0 (o); MS *m/z* (%) (70 eV): *m/z* =291.0 (100.0 %) [M+1], 292.0 (17.0 %) [M+2], 293.0 (6.0 %) [M+3], 294.0 (2.0 %) [M+4].

*2.2.9. Procedure for 2-isobutoxy-5-(4-methylthiazol-2-yl)benzoic acid (febuxostat mono acid-des acid impurity) (**12**)*
To a stirred solution of 2-(3-carboxy-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (**9,** 5.0 g, 0.016 mol.) in glacial acetic acid (30.0 mL) was added con. H_2SO_4 (2.5 mL) and water (20.0 mL) and reaction mixture was heated under 100-110°C for 24 h (until no more carbon dioxide is evolved). The reaction mass was chilled in an ice bath, made alkaline with 20.0 % sodium hydroxide solution (100.0 mL), and extracted with toluene (3 X 50.0 mL). The combine toluene layers were washed with water (2 X 50.0 mL), dried over anhydrous Na_2SO_4 (5.0 g), and the solvent was distillation off under vacuum at 50-55°C to give febuxostat mono acid-des acid impurity **12**.

[a] Refer structure formula of compound **11** in **Fig. 11** for numbering.

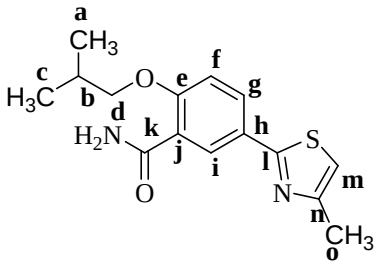
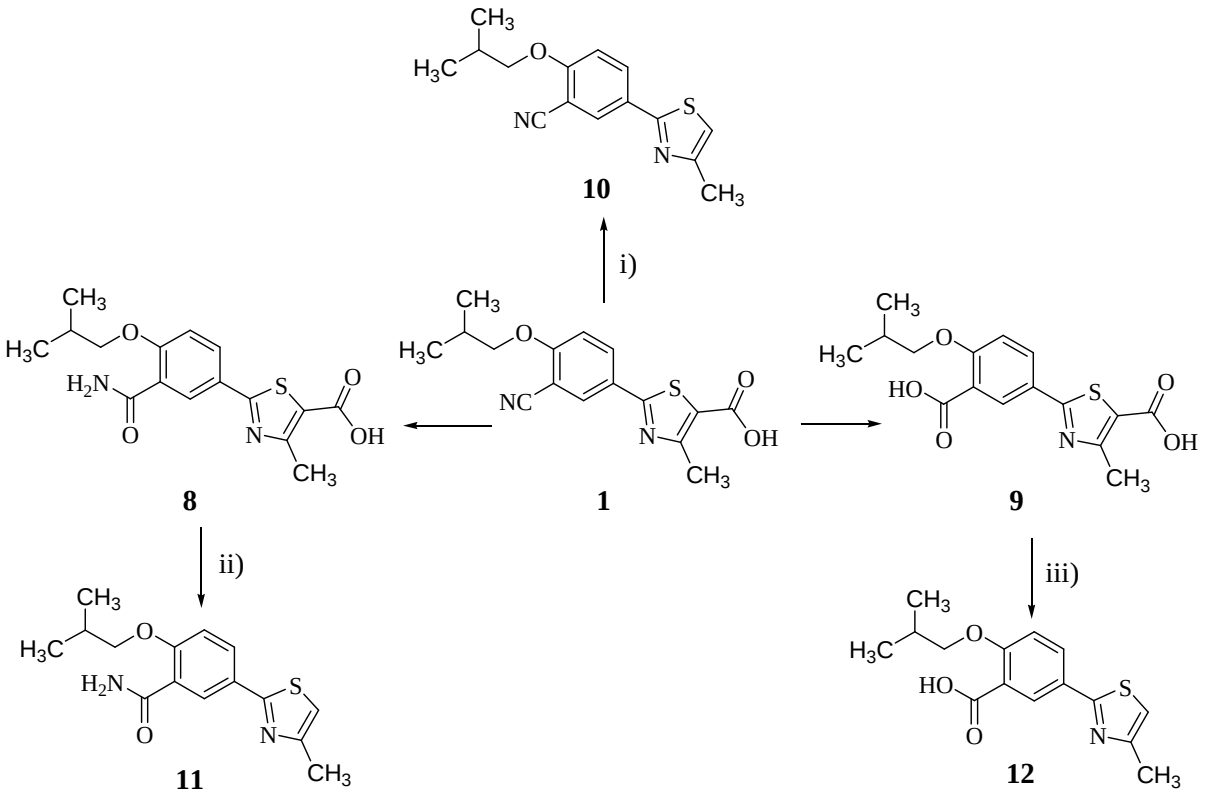


Fig. 11. 2-Isobutoxy-5-(4-methylthiazol-2-yl)benzamide (febuxostat amide-des acid impurity) (11), [*]Numbering has been assigned for ^{13}C NMR characterization

Yield 81.0 % (3.16 g), mp 165°C. Purity by HPLC: 95.995 % (7.349 min. retention time), Anal. Calcd for $C_{15}H_{17}NO_3S$: C, 61.83; H, 5.88; N, 4.81. Found: C, 61.80; H, 5.90; N, 4.84 %; IR (KBr) υ_{max} (in cm^{-1}): 3981.21, 3850.04, 3726.60, 3603.15, 3101.64, 2962.76, 2870.17, 2669.57, 2576.98, 2522.98, 2476.68, 2353.23, 2106.34, 1882.59, 1697.41, 1604.83, 1519.96, 1442.80, 1388.79, 1257.63, 1157.33, 1095.60, 1026.16, 918.15, 810.13, 663.53, 493.79; ^1H NMR (300 MHz, $CDCl_3$ or DMSO-d_6) δ_H (in ppm): 0.98-1.00 (d, 6H, $(C\underline{H}_3)_2$>CH-), 1.96-2.09 (m, 1H, $(CH_3)_2$>C<u>H</u>-), 3.85-3.87 (d, 2H, >CH-C<u>H</u>$_2$−), 7.18–8.14 (s, d, dd, 4H, Ar-<u>H</u>), 2.50 (s, 3H, -C<u>H</u>$_3$), 12.80 (s, 1H, -COO<u>H</u>); MS *m/z* (%) (70 eV): *m/z* =292.0 (100.0 %) [M+1], 293.0 (17.0 %) [M+2], 354.0 (7.0 %).



Scheme 4. Synthesis of febuxostat des acid impurities 9-12: Decarboxylation reaction: (i, ii and iii) glacial acetic acid, con. H_2SO_4, water, reflux (100-110°C)

3. High performance liquid chromatography (HPLC)

3.1. Reagents

Orthophosphoric acid (HPLC grade), Acetonitrile (HPLC grade), Methanol (HPLC grade), Water (Milli-Q).

3.2. Preparation of Buffer

0.5 mL of orthophosphoric acid was disolved in 1000.0 mL of water.

3.3. Preparation of mobile phase A

Buffer and acetonitrile were mixed in ratio of 60:40 v/v.

3.4. Preparation of mobile phase B
Acetonitrile and methanol were mixed in a ratio of 50:50 v/v.

3.5. Diluent
Water and acetonitrile were mixed in a ratio of 10:90 v/v.

3.6. Chromatographic conditions
Column: Hypersil BDS C-18, 5µ (150 X 4.6 mm) or equivalent
Pump mode: Gradient

3.7. Gradient program

Time (min.)	Mobile phase-A (% v/v)	Mobile phase (% v/v)
0.001	100.0	0.0
35.0	25.0	75.0
75.0	25.0	75.0
77.0	100.0	0.0
85.0	100.0	0.0
Flow rate :		1.5 mL/min.
Injection volume:		20.0 µL
Column temperature:		20.0 °C
Wave lenght:		317.0 nm
Run time:		85.0 min.

3.8. Preparation of solutions
3.8.1. Reference stock solution
2.0 mg of Febuxostat standard was accurately weighed into 100.0 mL clean dry volumetric flask, 20.0 mL of diluent was added, sonicated to dissolve and made up to volume with diluent. 5.0 mL of above solution was further diluted to 50.0 mL.

3.8.2. Reference solution
10.0 mL of reference stock solution was diluted into a 100.0 mL in a clean dry volumetric flask and made up to volume with diluent.

3.8.3. Sample solution
20.0 mg of sample was accurately weighed into a 100.0 mL clean dry volumetric flask, 20.0 mL of diluent was added and sonicated and made up to volume with diluent.

3.9. Evaluation of system suitability
20.0 µL of blank was injected into the chromatograph and chromatogram was recorded. 20.0 µL of sample injected reference solution (six replicate) was injected into the chromatograph and the chromatogram was recorded. % RSD for six replicate injections of reference solution is not more than 5.0.

3.10. Procedure
20.0 µL of blank was injected into the chromatograph and chromatogram was recorded. 20.0 µL of sample solution was injected into the chromatograph and chromatogram was recorded. Blank chromatograms were examined for any peaks due to blank and corresponding peaks were disregard if observed in the chromatogram of the sample solution.

Macleods Pharmaceuticals Ltd
R and D Centre , Mumbai

Sample Name: Febuxostat
Sample ID : Spike Sample
Tray No : 1
Vail No : 6
Inj Vol : 20 uL
:

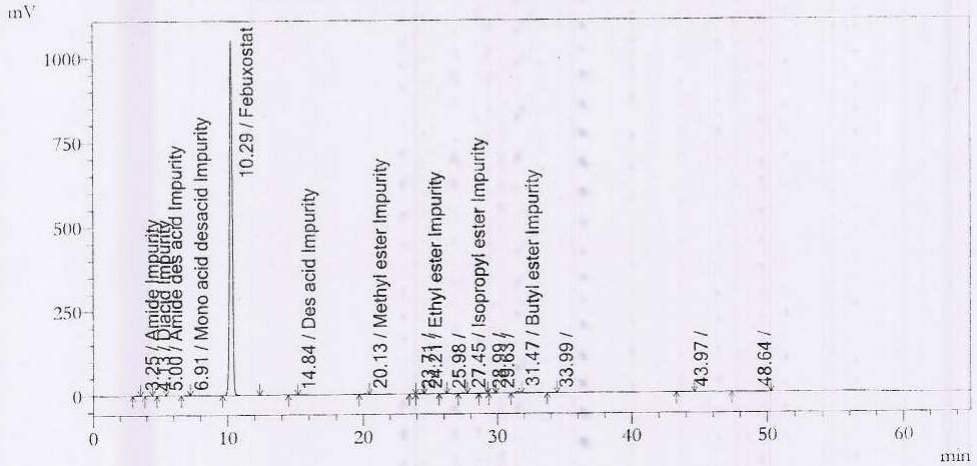
Data Filename : 011.lcd
Analysis Method : RS_off.lcm
Batch Filename : 230312.lcb
Date Acquired : 3/23/2012 12:34:39 PM
:

Data Description: Spike Sample
PRL/I/1045/03/42/12
Buffer--0.05%OPA
MPA-Buffer:ACN(60:40)
MPB--ACN:Methanol(1:1)
Diluent--Water:ACN(1:9)
Flow-1.3ml/Min
Inj Volume-10ul
Temp-25°C
Column--Hyoersil BDS C-18(150X4.6)5um(02COXH002
Gradent--

0.01	Pumps	Pump B
30.00	Pumps	Pump B
55.00	Pumps	Pump B
57.00	Pumps	Pump B
65.00	Pumps	Pump B
65.01	Controller	Stop

Data flie path:-C:\LabSolutions\Data\Project1\FEBUXOSTAT\Data\220312\011.lcd
Method path:-C:\LabSolutions\Data\Project1\FEBUXOSTAT\Method\RS_off.lcm

Chromatograms
Febuxostat C:\LabSolutions\Data\Project1\FEBUXOSTAT\Data\220312\011.lcd



Detector A Ch1 317nm

Peak#	Ret. Time	Area	Area %	Name
1	3.251	20793	0.137	Amide Impurity
2	4.132	15716	0.103	Diacid Impurity
3	5.004	16087	0.106	Amide des acid Impurity
4	6.914	14916	0.098	Mono acid desacid Impurity
5	10.287	14930855	98.236	Febuxostat
6	14.837	15674	0.103	Des acid Impurity
7	20.125	41263	0.271	Methyl ester Impurity
8	23.706	1931	0.013	
9	24.214	24040	0.158	Ethyl ester Impurity
10	25.981	479	0.003	
11	27.448	20331	0.134	Isopropyl ester Impurity
12	28.995	2886	0.019	
13	29.628	4754	0.031	
14	31.473	69993	0.461	Butyl ester Impurity
15	33.993	3275	0.022	
16	43.973	10541	0.069	
17	48.645	5506	0.036	
Total		15199039	100.000	

Fig. 12. Typical chromatograph of febuxostat crystal A spiked with 9.0 impurities 4-12

RESULTS AND DISCUSSION

In the synthesis of Febuxostat (scheme-1) in the step-3 tech grade Febuxostat is purified using methanol as asolvent. Formation of methylester impurity **4** is attributted to the esterification of Febuxostat with methanol. Methyl ester impurity **4** was indipedently prepared by reacting Febuxostat with methanol in the presence of catalytic amount of conc sulfuric acid (Scheme 2).

Ethyl ester **5** one of the intermediates in the synthetic sequence of Febuxostat can be carry forward intact in the API as a related substance. Ethyl ester **5** was made in an alternate process for the esterification of Febuxostat with ethanol in the presence of catalytic amount of conc sulfuric acid.

Formation of isopropyl ester **6** was attributted to a posible trans esterification in step-1 of Febuxostat synthesis **6** isopropyl alcohol was used as a solvent in the condensation of thioamide derivative **2** with 2-chloroethylaceto acetate. Febuxostat was subjected to esterification using isopropyl alcohol to prepare a standard of isopropyl ester of **6**.

Febuxostat butyl ester impyurity **7** is a process related impurity, resulting from the isolation of Febuxostat **1** (acidic pH adjustment) in step-2, wherein 1-butanol used as a solvent. Impurity **7** was prepared indipendently from the reaction of acid **1** with 1-butanol utilizing same reaction conditions.

Formation of Febuxostat amide impurity **8** and Febuxostat diacid impurity **9** was resulted to form the alkaline hydrolysis of Fubuxostat ethyl ester **5** in the step-2 of Febuxostat synthesis. Bothe related compounds **8** and **9** were indipendently were prepared from the sodium hydroxide mediated hydrolysis of cyano ester derivative-4.

Febuxostat desacid **10**, Febuxostat amide desacid **11** and Febuxostat monoacid desacid **12** impurities were process related impurities resulting from hydrolysis of nitrile function accompained by decarboxylation as by products in the step-2 of Febuxostat synthesis. These impurities **10-12** were synthesized indipently from **1**, **8** and **9**, respectively by decarboxylation reaction (scheme-4).

These nine impurities were characterized and confirmed by ^{1}H and ^{13}C NMR, Mass, IR spectroscopic data and elemental analysis.

CONCLUSION

In conclusion, nine process related impurities (related substances were identified in the commercial synthesis of an active pharmaceutical ingredient, Febuxostat. They were identified and fully characterized based on their spectral data and elemental analysis.

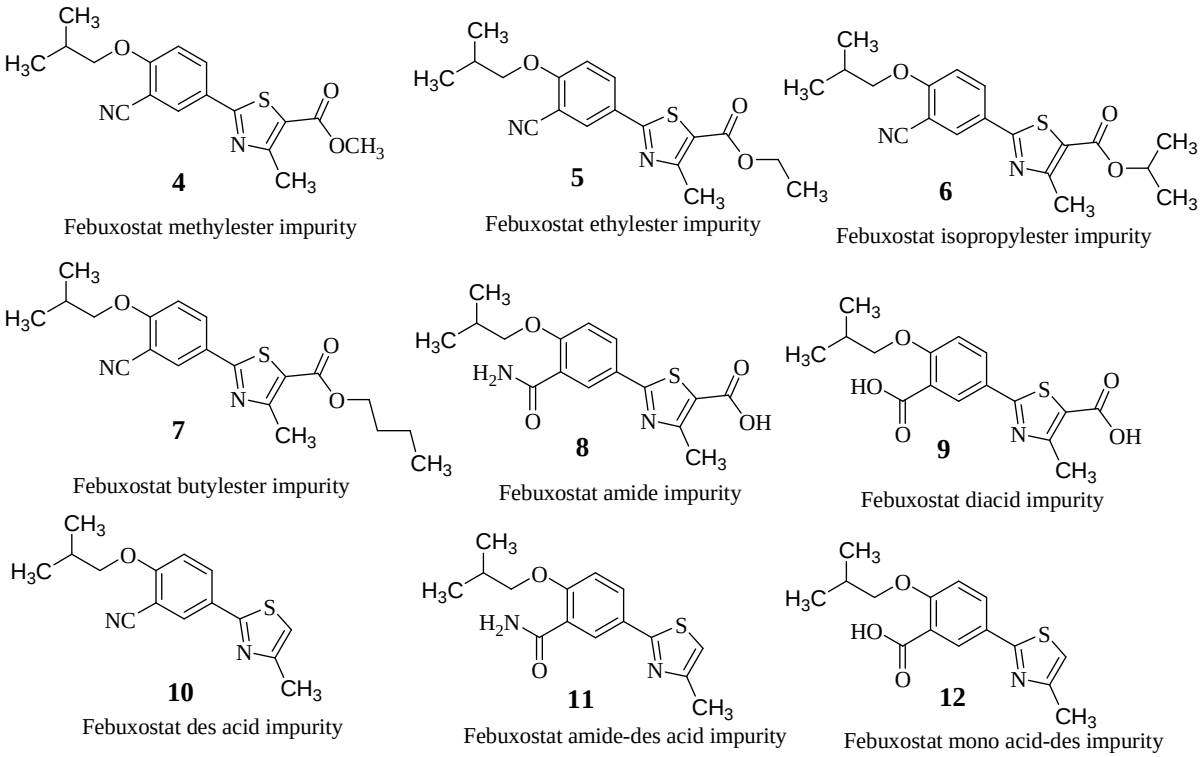


Fig. 13. Structure of critical process related impurities 4-12 of febuxostat

Acknowledgments

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