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

Venkateswara Rao Vallu, Krunal Girishbhai Desai, Sandip Dhaya Patil, Rajendra Agarwal, Pratap Reddy Padi and Mahesh Reddy Ghanta*

Process Research Laboratory-I, Research & Development Centre, Macleods Pharmaceuticals Ltd, G-2, Mahakali Caves Road, Shantinagar, Andheri (East), Mumbai, Maharashtra, India

Department of Chemistry, Pacific University, Pacific Hills, Airport Road, Pratap Nagar Extension, Debari, Udaipur, Rajasthan, India

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; impurities; synthesis; characterization, gout

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 research, 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 requirements 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.

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 impurity 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 ($\delta_{H}, \delta_{C}$) are reported in ppm relative to TMS. The FT-IR spectra ($\nu_{max}$ 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. Caled for C$_{16}$H$_{15}$NSO$_2$: C, 62.77; H, 5.85; N, 8.13; IR (KBr) $\nu_{max}$ (in cm$^{-1}$): 3394.83, 3070.78, 2974.33, 2840.59, 2808.56, 2225.93, 1894.16, 1820.86, 1708.99, 1605.69, 1512.24, 1469.97, 1325.26, 1265.82, 1136.38, 1051.66, 943.79; $^1$H NMR (300 MHz, CDCl$_3$): 6.75-7.04 (m, 3H, Ar-H), 2.15-2.28 (m, 1H, (CH$_2$)$_2$), 2.15-2.28 (m, 1H, (CH$_2$)$_2$), 1.38-1.42 (t, 3H, -OCH$_3$); $^{13}$C NMR (300 MHz, DMSO–D$_6$): 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.

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 (I).

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_{2}O_{3}S: C, 60.74; H, 5.10; N, 8.85. Found: C, 60.70; H, 5.11; N, 8.87 %; \text{IR (KBr) } \nu_{\text{max}} \text{ (in cm}^{-1}) \text{: } 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; \text{1H NMR (300 MHz, CDCl}3 \text{or DMSO–d}6 \text{)} \delta_{H} \text{ (in ppm): } 1.00-1.02 (d, 6H, (CH}_{3}2\text{-CH-}), 2.49-2.50 (m, 1H, (CH}_{3}2\text{-CH-}), 3.97-3.99 (d, 2H, -CH-CH}_{2}-), 7.33–8.25 (d, dd, 3H, Ar-H), 2.64 (s, 3H, -CH}_{3}), 13.39 (s, 1H, -COOH); \text{13C NMR (300 MHz, DMSO–d}6 \text{)} \delta_{C} \text{ (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), 101.7 (f), 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 %).

 Refer structure formula of compound (I) in Fig. 4 for numbering.

![Fig. 4. 2-(3-Cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (I), *Numbering has been assigned for 13C NMR characterization.](image)

**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_{2}SO_{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_{2}SO_{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\textsubscript{18}H\textsubscript{17}N\textsubscript{2}O\textsubscript{3}: C, 61.80; H, 5.49; N, 8.48. Found: C, 61.83; H, 5.44; N, 8.43 %; IR (KBr) \(\nu_{\text{max}}\) (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-\(d_{6}\)) \(\delta_{H}\) (in ppm): 1.01-1.03 (d, 6H, \(\text{-CH\textsubscript{3}}\)), 2.07-2.20 (m, 1H, \(\text{-CH}_{2}\)), 2.2-2.34 (d, 2H, \(\text{>CH-CH}_{2}\)), 6.92-8.10 (m, 3H, Ar-H), 2.69 (s, 3H, \(-\text{OCH}_{3}\)). MS \(m/z\) (%): 100 (10.0 %); 287 (14.0 %), 280 (20.0 %), 270 (15.0 %), 232 (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\textsubscript{20}H\textsubscript{20}N\textsubscript{2}O\textsubscript{3}: C, 62.77; H, 5.85; N, 8.13. Found: C, 62.81; H, 5.83; N, 8.31 %. IR (KBr) \(\nu_{\text{max}}\) (cm\(^{-1}\)): 3394.83, 3070.78, 2974.33, 2874.03, 2804.59, 2588.56, 2225.93, 1894.16, 1820.86, 1708.99, 1604.83, 1512.44, 1427.37, 1386.41, 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-\(d_{6}\)) \(\delta_{H}\) (in ppm): 1.09-1.11 (d, 6H, \(\text{-CH\textsubscript{3}}\)), 2.15-2.28 (m, 1H, \(\text{-CH}_{2}\)), 3.90-3.92 (d, 2H, \(\text{>CH-CH}_{2}\)), 7.01-8.19 (m, 3H, Ar-H), 2.78 (s, 3H, \(-\text{OCH}_{3}\)), 4.33-4.40 (q, 2H, \(\text{-OCH}_{2}-\text{CH}_{3}\)). \(^{13}C\) NMR (300 MHz, DMSO-\(d_{6}\)) \(\delta_{C}\) (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 (l), 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 (100.0 %); 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), \(^{1}H\) NMR 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), \(^{1}H\) NMR 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\textsubscript{19}H\textsubscript{20}N\textsubscript{2}O\textsubscript{3}: C, 63.66; H, 6.19; N, 7.82. Found: C, 63.67; H, 6.23; N, 7.84 %. IR (KBr) \(\nu_{\text{max}}\) (cm\(^{-1}\)): 3614.72, 3390.97, 3210.93, 3070.78, 2974.33, 2955.04, 2874.03, 2804.43, 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-\(d_{6}\)) \(\delta_{H}\) (in ppm): 1.00-1.03 (d, 6H, \(\text{-CH\textsubscript{3}}\)), 2.06-2.20 (m, 1H, \(\text{-CH}_{2}\)), 3.81-3.83 (d, 2H, \(\text{-CH}_{2}\)), 6.92-8.10 (m, 3H, Ar-H), 2.68 (s, 3H, \(-\text{OCH}_{3}\)), 5.09-5.18 (m, 1H, \(-\text{OCH}_{2}\)), 1.28-1.30 (d, 6H, \(-\text{OCH}_{2}\)), \(^{13}C\) NMR (300 MHz, DMSO-\(d_{6}\)) \(\delta_{C}\) (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
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 C20H24N2O3S: C, 64.49; H, 6.49; N, 7.52. Found: C, 64.53; H, 6.53; N, 7.50 %; IR (KBr) \( \nu_{\text{max}} \) (in cm\(^{-1}\)): 3375.54, 3205.80, 3174.94, 3120.93, 3082.35, 3063.06, 3032.20, 2966.62, 2931.90, 2874.03, 2834.21, 2764.81, 2737.80, 2233.64, 2209.89, 1940.45, 1898.02, 1836.29, 1759.14, 1701.27, 1604.83, 1570.11, 1512.24, 1454.38, 1392.65, 1369.50, 1319.35, 1276.92, 1168.90, 1099.46, 1045.45, 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-\(d_6\)) \( \delta \) (in ppm): 1.01-1.03 (d, 6H, -(CH\(_3\))\(_2\)-CH-), 2.07-2.29 (m, 1H, -(CH\(_3\))\(_2\)-CH-), 3.82-3.84 (d, 2H, >CH-CH\(_2\)-), 6.92–8.11 (d, dd, 3H, Ar-H), 2.69 (s, 3H, -CH\(_3\)), 4.21-4.25 (t, 2H, -OCH\(_2\)-CH\(_2\)-CH\(_2\)-CH\(_3\)), 1.62-1.71 (m, 2H, -OCH\(_2\)-CH\(_2\)-CH\(_2\)-CH\(_3\)), 1.34-1.50 (m, 2H, -OCH\(_2\)-CH\(_2\)-CH\(_2\)-CH\(_3\)), 0.89-0.94 (t, 3H, -OCH\(_2\)-CH\(_2\)-CH\(_2\)-CH\(_3\)).

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].

\( ^{13}C \) NMR (300 MHz, DMSO–\(d_6\)) \( \delta \) (in ppm) (Position): 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].

*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*](image)

### Scheme 2 Synthesis of febuxostat ester impurities: (i) Esterification reaction: Con. H\(_2\)SO\(_4\), 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 respective 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\(_2\)CO\(_3\) powder (5.0 g) and drop wise 30.0 % H\(_2\)O\(_2\) (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 to 1-2 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\(_2\)O\(_4\)S: C, 57.47; H, 5.43; N, 8.38. Found: C, 57.50; H, 5.40; N, 8.35 %. IR (KBr) \( \nu_{\text{max}} \) (in cm\(^{-1}\)): 3402.54, 3290.67, 3186.51, 3066.92, 2970.48, 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, 1649.81, 1469.81, 1427.37,

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1377.22, 1323.21, 1292.35, 1273.06, 1257.63, 1226.77, 1161.19, 1118.75, 1099.46, 1041.60, 1014.85, 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-\(d_6\)) \(\delta_H\) (in ppm): 0.99-1.01 (d, 6H, (CH\(_3\))\(_2\)>CH-), 2.06-2.19 (m, 1H, (CH\(_3\))\(_2\)>CH-), 3.96-3.98 (d, 2H, >CH-CH\(_3\)-), 7.25–8.34 (d, dd, 3H, Ar-H); \(^13\)C NMR (300 MHz, DMSO-\(d_6\)) \(\delta_C\) (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 (b), 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 (11.0 %) [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](https://example.com/fig8.png)

Fig. 8. 2-(3-Carbamoyl-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (febuxostat amide impurity) (8), \(^a\)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 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\(_2\)S: C, 57.30; H, 5.11; N, 4.18. Found: C, 57.33; H, 5.08; N, 4.20 %; IR (KBr) \(\nu_{max}\) (in cm\(^{-1}\)):

- 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, 1323.06, 1292.35, 1273.06, 1257.63, 1226.77, 1161.19, 1118.75, 1099.46, 1041.60, 1014.85, 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\(_3\)) \(\delta_H\) (in ppm): 0.99-1.01 (d, 6H, (CH\(_3\))\(_2\)>CH-), 2.06-2.19 (m, 1H, (CH\(_3\))\(_2\)>CH-), 3.96-3.98 (d, 2H, >CH-CH\(_3\)-), 7.22–8.22 (d, dd, 3H, Ar-H), 2.66 (s, 3H, -CH\(_3\)), 13.04 (s, 2H, -COOH); \(^13\)C NMR (300 MHz, DMSO-\(d_6\)) \(\delta_C\) (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 (b), 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](https://example.com/fig9.png)

Fig. 9. 2-(3-Carboxy-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (febuxostat diacid impurity) (9), \(^a\)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\(_2\)SO\(_4\) (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\(_2\)SO\(_4\) (5.0 g), and solvent was removed by distillation under vacuum at 50-55°C to give febuxostat des acid impurity 10.

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305
Scheme 3 Synthesis of febuxostat amide 8 and febuxostat diacid 9 impurities: (i) H$_2$O$_2$ (30.0 %), DMSO, anhydrous K$_2$CO$_3$, 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$_2$O$_2$: C, 66.15; H, 5.92; N, 10.29. Found: C, 66.18; H, 5.95; N, 10.33 %; IR (KBr) $\nu_{\text{max}}$ (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, 1526.24, 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$_6$) $\delta$ (in ppm): 1.00-1.02 (d, 6H, (CH$_3$)$_6$-), 3.97-3.99 (d, 2H, >CH-CH$_2$-), 7.33-8.20 (s, d, dd, 4H, Ar-H), 2.50 (s, 3H, -CH$_3$); $^{13}$C NMR (300 MHz, DMSO-d$_6$) $\delta$ (in ppm) (Position): 164.9 (I), 162.0 (e), 154.0 (m), 133.3 (g), 131.1 (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 %).

Fig. 10. 2-Isobutoxy-5-(4-methylthiazol-2-yl)benzonitrile (febuxostat des acid impurity) (10), a 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-carboxamidoyl-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid (8, 5.0 g, 0.016 mol) in glacial acetic acid (30.0 mL) was added conc. H$_2$SO$_4$ (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$_2$SO$_4$ (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$_{16}$N$_2$O$_2$: C, 66.14; H, 5.65; N, 9.65. Found: C, 66.08; H, 6.20; N, 9.62 %; IR (KBr) $\nu_{\text{max}}$ (in cm$^{-1}$): 3398.69, 3290.67, 3263.66, 3186.51, 2966.62, 2924.18, 2870.17, 2777.59, 2507.54, 2468.97, 2360.95, 2291.51, 2222.07, 2137.20, 2036.90, 1928.88, 1735.99, 1705.13, 1683.24, 1526.24, 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$_6$) $\delta$ (in ppm): 1.00-1.02 (d, 6H, (CH$_3$)$_6$-), 3.97-3.99 (d, 2H, >CH-CH$_2$-), 7.22–8.30 (s, dd, 2H, Ar-H), 2.50 (s, 3H, -CH$_3$); $^{13}$C NMR (300 MHz, DMSO-d$_6$) $\delta$ (in ppm) (Position): 164.9 (I), 162.0 (e), 154.0 (m), 133.3 (g), 131.1 (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 %).

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 conc. H$_2$SO$_4$ (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$_2$SO$_4$ (5.0 g), and the solvent was distillation off under vacuum at 50-55°C to give febuxostat mono acid-des-acid impurity 12.
"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$_{3}$S: C, 61.83; H, 5.88; N, 4.81. Found: C, 61.80; H, 5.90; N, 4.84 %. IR (KBr) $\nu_{\text{max}}$ (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; $^1$H NMR (300 MHz, CDCl$_3$ or DMSO-$d_6$) $\delta_H$ (in ppm): 0.98-1.00 (d, 6H, (CH$_3$)$_2$>CH-), 1.96-2.09 (m, 1H, (CH$_3$)$_2$>CH-), 3.85-3.87 (d, 2H, >CH-CH$_2$−), 7.18–8.14 (s, d, dd, 4H, Ar-H), 2.50 (s, 3H, -CH$_3$), 12.80 (s, 1H, -COOH); 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$_2$SO$_4$, 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 dissolved 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

<table>
<thead>
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<th>Time (min.)</th>
<th>Mobile phase-A (% v/v)</th>
<th>Mobile phase (% v/v)</th>
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<tr>
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</tbody>
</table>

Flow rate: 1.5 mL/min.
Injection volume: 20.0 µL
Column temperature: 20.0 ºC
Wave length: 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.
RESULTS AND DISCUSSION

In the synthesis of Febuxostat (scheme-1) in the step-3 tech grade Febuxostat is purified using methanol as a solvent. Formation of methylester impurity 4 is attributed to the esterification of Febuxostat with methanol. Methyl ester impurity 4 was independently 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 attributed to a possible transesterification in step-1 of Febuxostat synthesis 6 isopropyl alcohol was used as a solvent in the condensation of thioamide derivative 2 with 2-chloroethylacetoacetate. Febuxostat was subjected to esterification using isopropyl alcohol to prepare a standard of isopropyl ester of 6.

Febuxostat butyl ester impurity 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 independently 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 Febuxostat ethyl ester 5 in the step-2 of Febuxostat synthesis. Both related compounds 8 and 9 were independently 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 accompanied by decarboxylation as by products in the step-2 of Febuxostat synthesis. These impurities 10-12 were synthesized independently from 1, 8 and 9, respectively by decarboxylation reaction (scheme-4).

These nine impurities were characterized and confirmed by 1H and 13C 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

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REFERENCES