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Structural identification, characterization and synthesis of impurities of Naproxcinod

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Abstract: Five potential process related impurities were detected during the synthesis of anti-inflammatory drug Naproxcinod (1). Simple high performance liquid chromatography and liquid chromatography-mass spectrometry methods were used for the detection of these impurities. These impurities were synthesized and co-injected with naproxcinod to confirm the retention times in HPLC. Based on the spectral data (IR, NMR and MS), the structures of these impurities were characterized as (*S*)-2-(6-methoxynapthalen-2-yl) propanoic acid (impurity I), (*S*)-4-chlorobutyl 2-(6-methoxyanpthalen-2-yl) propanoate (impurity II), (*S*)-4-bromobutyl 2-(6-methoxyanpthalen-2-yl) propanoate (impurity III), (*S*)-4-hydroxybutyl 2-(6-methoxyanpthalen-2-yl) propanoate (impurity IV), (2*S*,2*S*)-butane-1,4-diyl bis (2-(6-methoxynapthalen-2-yl) propanoate) (impurity V). Formation of these impurities was discussed.

Keywords: Naproxcinod, Impurities, Spectroscopy, Identification, Characterization and synthesis

Introduction

Naproxcinod belongs to a unique class of ant-inflammatory drugs known as Cyclooxygenase Inhibiting Nitric Oxide Donators (CINODs). [1-6] Naproxcinod is developed by the French pharmaceutical company NiCoX, is most advanced investigational drug and used as anti-inflamatory agent, Naproxcinod is the first class of NSAIDs known as cyclooxygenase (COX) inhibiting NO donars (CINODs), it is a derivative of naproxen with similar anti-inflamatory activity, but less gastrointestinal toxicity. Naproxcinod is chemically designated as 4-nitroxybutyl (2S)-2-(6-methoxynapthalen-2-yl) propanoate. Its molecular formula is $C_{18}H_{21}NO_6$ and molecular weight is 347.36 [7-8]. Challenging task for a process chemist is to identify all the impurities formed during the synthesis of active pharmaceutical ingredient

and the impurities formed are need to be controlled or washed off in order to meet the requirement of various regulatory agencies. Thus, in the present study we described the formation, identification, synthesis, and characterization of impurities formed in the preparation of naproxcinod. During the analysis of different laboratory batches of naproxcinod, five impurities were detected consistently in almost all the batches, whose area percentage ranged from 0.05 to 0.2 %, by reverse phase LC. The impurity profile study has to be carried out for any final product as per the regulatory requirements to identify and characterize all the unknown impurities. Naproxcinod can be synthesized from Naproxen and 4-nitoxy butanol or 4-nitroxy butyl halide/tosylate in one step,[9-12] it can be prepared from Naproxen and 1,4-dihalo butane or 4-halo butanol, and the corresponding halo intermediate was converted to naproxcinod by using silver nitrate.[13-16]

This paper not only describes the identification, synthesis and characterization of impurities present in naproxcinod but also explains the formation of these impurities. The impurity profile study by HPLC, detection of mass by LC-MS, synthesis and characterization of the detected impurities were not reported till date to the best of our knowledge.

Results and discussions

Detection of impurities I, II, III, IV and V

All these impurities were detected in reverse phase high performance liquid chromatography. The target impurities under study are marked as Imp-I, Imp-II Imp-III, Imp-IV and Imp-V. All these impurities were synthesized in significant quantities for spectroscopic studies and analytical method validations. Impurities I, II, III and IV are polar impurities and V is non-polar with respect to naproxcinod. The prepared impurities were co-injected with naproxcinod to confirm the identity of the impurities based on retention time matching. All the impurities were well resolved from naproxcinod and each other. Retention times in LC, structures of these impurities and naproxcinod are shown in Table 1.

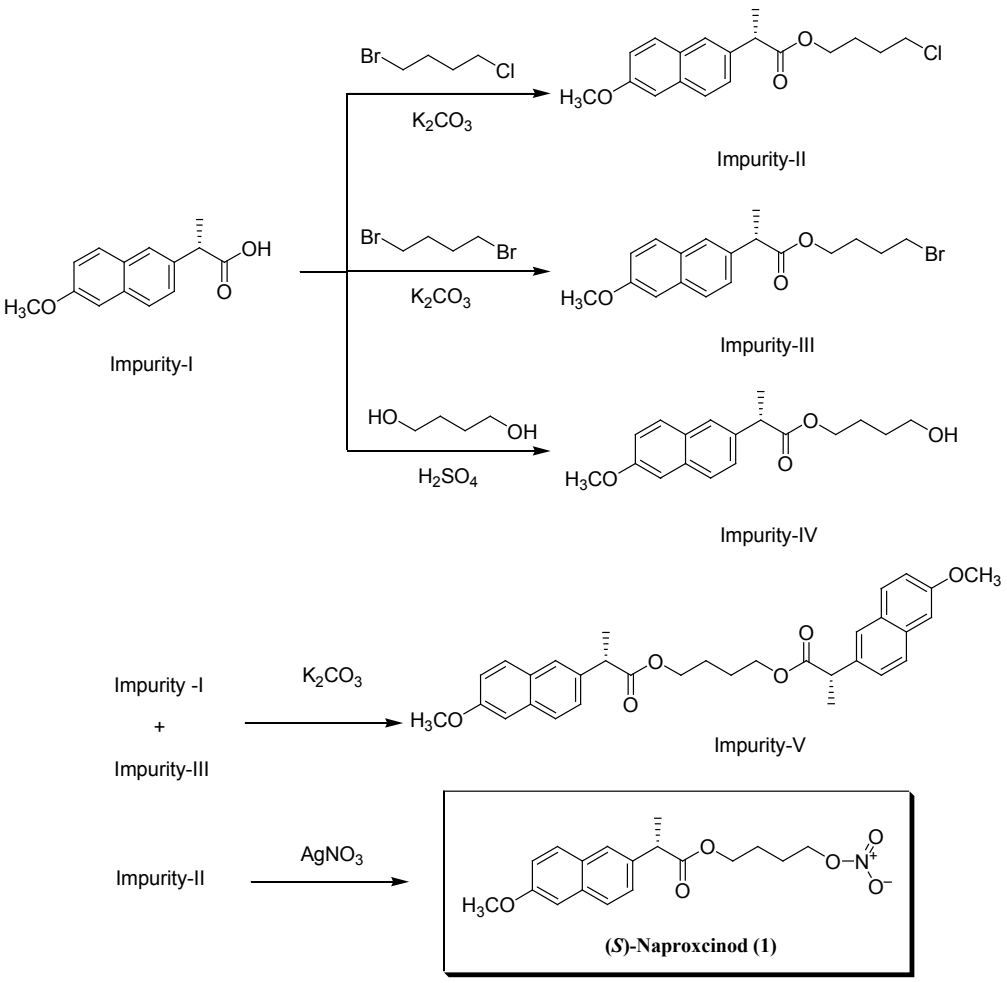
Formation of impurities

One of the key starting material and intermediates used in the synthesis of naproxcinod is impurity I and II respectively. During the synthesis of impurity II, traces of 1,4-dibromo butane present in the 1-bromo-4-chloro butane will facilitate the formation of impurity III. During the final step of naproxcinod if the reaction contaminated with traces of water can lead the formation of impurity IV. During synthesis of key intermediate (impurity II) of the naproxcinod, contained excess of naproxen (impurity I) reacts with impurity II and leading to the formation of impurity dimer impurity V. The scheme for the formation of impurities is shown in Scheme 1. The rationalization given above clearly indicates that these impurities are process-related.

Structural elucidation of naproxcinod and its impurities

Structural elucidation of naproxcinod

ESI mass spectrum of naproxcinod exhibited molecular ion peak as sodium adduct at m/z 370 and fragment pattern was observed with ion m/z 302, 185 atomic mass units (amu) in positive mode. In ^{1}H NMR of naproxcinod, the signals at 4.29 ppm corresponding to CH_2 which is linked with ONO_2 and a signal at 4.09 ppm corresponding to acid linked CH_2 ($RCOOCH_2$-) was observed. In IR spectrum, band at 1731 cm^{-1} corresponding to acid C=O, a sharp band at 1627 cm^{-1} corresponding to $–NO_2$ and a band at 1280 cm^{-1} corresponding to acid C-O linkage was observed.



Scheme 1: Synthesis of Naproxcinod and its Impurities (II, III, IV and V)

The DEPT spectra (Fig 2) displayed four negative (down) signals due to four methylene groups and nine positive (up) signals due to the presence of two methyl groups and rest are due to the methine groups. Based on the above spectral data the molecular formula of naproxcinod conformed as $C_{18}H_{21}NO_6$ and corresponding structure was conformed as 4-nitroxybutyl (2S)-2-(6-methoxynapthlen-2-yl) propanoate.

Structural elucidation of impurity I

The spectral data of impurity I are compared with impurity V.

The ESI mass spectrum of impurity I exhibited molecular ion at m/z 230 amu. In ^{1}H NMR of this impurity, the signals at 7.67 ppm corresponding to acid OH and signal at 3.9 ppm corresponding to CH territory carbon were observed. The DEPT spectrum displayed nine positive signals due to the presence of two methyl and the rest are due to methine groups (one in the aliphatic and the rest six in aromatic region). The ^{13}C NMR spectrum displayed 14 signals due to the presence of fourteen carbons. The FT-IR spectrum displayed a sharp band at 1685 cm^{-1} corresponding to acid C=O functional group, which was supported by the appearance of quaternary carbon signal at 180.5 ppm in

^{13}C NMR. Based on the above spectral data the molecular formula of impurity I could be $C_{14}H_{14}O_3$. This molecular formula matched well with the molecular ion observed at 230 amu in the EI mass spectrum. The specific optical rotation 56° (c = 1 % $CHCl_3$) was observed.

The data obtained from the spectral studies can be rationalized in terms of impurity I having the molecular formula $C_{14}H_{14}O_3$ and the corresponding structure was characterized as (*S*)-2-(6-methoxynapthalen-2-yl) propanoic acid.

Structural elucidation of impurity II, III and IV

The spectral data of impurity II, III and IV are compared with that of naproxcinod. The EI mass spectra of the impurities II and III exhibited molecular ion at m/z 320 and 364 amu with a characteristic M+ 2 molecular ion at 322, 366 with a ~1:3 and 1:1 intensity respectively, which is 27, 45 amu less than that of naproxcinod (*m*/*z* 347) respectively. The even molecular ion in the mass spectrum indicates the obscene or even number of nitrogen atoms. This observation indicates that the $–ONO_2$ atom in naproxcinod is replaced by chlorine, bromine as observed form the mass spectral data. The EI mass spectra of the impurity IV displayed molecular ion at *m*/*z* 302, which is 45 amu less that of naproxcinod (*m*/*z* 347). In addition to this observation, the characteristic absorption band at around 1627 cm^{-1} due to stretching of $-O-NO_2$ in the FT-IR spectrum of naproxcinod was absent in the spectrum of impurity II, III and IV and a strong band at 3396 cm^{-1} corresponding OH observed for impurity IV.

It is interesting to note that impurities II, III and IV have same skeletal system as evident by the number of NMR signals. The ^1H, ^{13}C NMR chemical shift of methylene carbon adjacent to chlorine, bromine, and hydroxyl group appeared at 4.10, 4.01, 4.07 ppm and 44.3, 32.9, 37.7 ppm for impurities II, III and IV, respectively. Thus the impurities II, III and IV structure can be explained in terms of removal of $–ONO_2$ from naproxcinod and addition of chlorine, bromine, and hydroxyl group in naproxcinod respectively. From the above spectral data the molecular formula of impurity II was confirmed as $C_{18}H_{21}ClO_3$ and the corresponding structure was characterized as (*S*)-4-chlorobutyl 2-(6-methoxyanpthalen-2-yl) propanoate; impurity III was confirmed as $C_{18}H_{21}BrO_3$ and the corresponding structure was characterized as (*S*)-4-bromobutyl 2-(6-methoxyanpthalen-2-yl) propanoate; impurity IV was confirmed as $C_{18}H_{22}O_4$ and the corresponding structure was characterized as (*S*)-4-hydroxybutyl 2-(6-methoxyanpthalen-2-yl) propanoate.

Structural elucidation of impurity V

The spectral data of impurity V are compared with that of naproxcinod.

The EI mass spectrum exhibited molecular ion at 514 amu, which is 167 amu more than that of naproxcinod. The even molecular ion in the mass spectrum indicates the obscene or even number of nitrogen atoms. Interestingly, the absent of characteristic 302 fragment which was due to the cleavage of $–NO_2$ in naproxcinod and presence of 285 fragment was observed in the mass spectrum of impurity V. In addition to this observation, the characteristic absorption band at around 1647 cm^{-1} due to $–ONO_2$ stretching in the FT-IR spectrum of naproxcinod was absent in the spectrum of impurity V. Further, an additional signal in the ^1H and ^{13}C spectra in the aromatic region indicate the impurity V contain an additional methoxynapthalene propanoate moiety. Thus the impurities V structure can be explained in terms of removal of $O-NO_2$ and addition of additional methoxynapthalene propanoate moiety. In addition to this observation, in the DEPT spectrum disappearance of characteristic signal at 72 ppm related to $–CH_2-ONO_2$ and

Fig.1. DEPT spectrum of impurity V in $CDCl_3$, 200 MHz

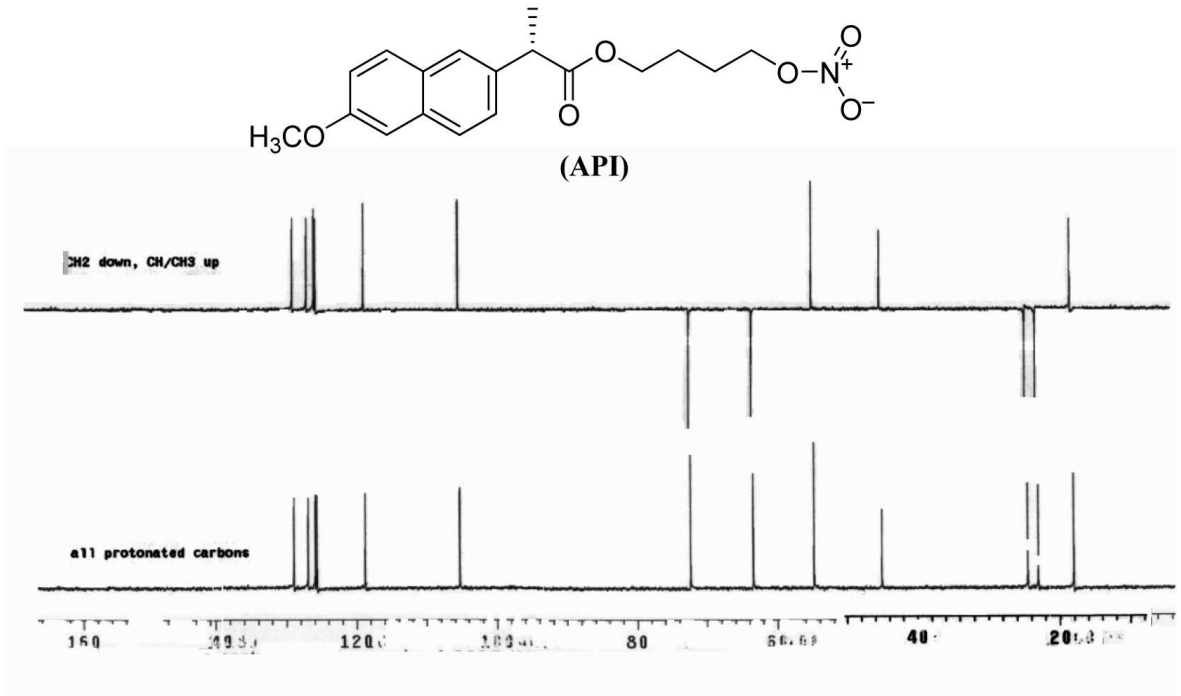


Fig.2. DEPT spectrum of naproxcinod (API) in $CDCl_3$, 400 MHz

appearance only two methelene signals at 64 and 24 ppm clearly indicating the molecular structure is dimer with having a center of symmetry (Fig 1and 2).

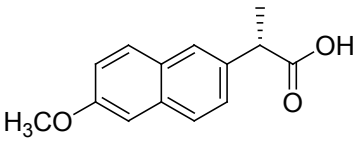
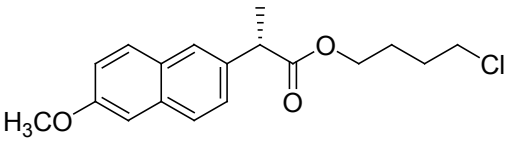
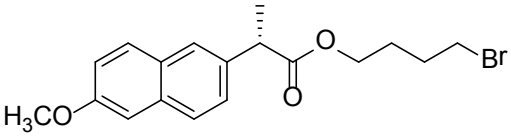
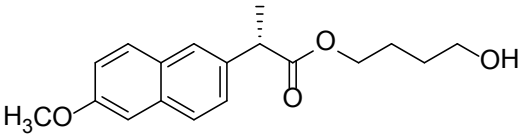
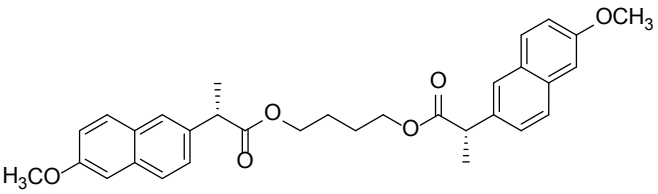
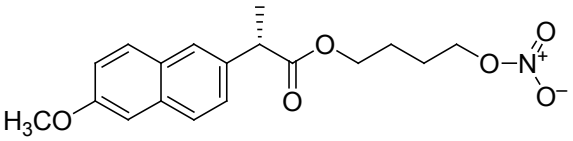
Based on the above spectral data the molecular formula of impurity V was confirmed as $C_{32}H_{34}O_6$ and the corresponding structure was characterized as (2*S*,2*S*)-butane-1,4-diyl bis (2-(6-methoxynapthalen-2-yl) propanoate).

Experimental procedure:

Synthesis of Impurity-II and III:

Naproxen (30.0 gm 0.13 mol) and acetonitrile (600 ml) are charged into a round bottom flask and stirred for 5-10 min at ambient temperature.

Table-1

S.No.	Name	Structure	Ret. Time (min.)*
01	Impurity-I		5.30
02	Impurity-II		24.37
03	Impurity-III		25.94
04	Impurity-IV		7.09
05	Impurity-V		38.31
06	API		22.70

*Column: symmetry shield RP-18, 100x4.6x3.5µm, flow rate: 1.0ml/min, λ: 230nm, injection vol: 10µL; mobile phase-A: Buffer/ACN (65:35), Mobile phase B: ACN/Buffer (75:25); Buffer: 0.02M potassium dihydrogen phosphate and adjust the pH to 2.2 with dilute phosphoric acid;

K_2CO_3 (21.5 gm) is added and the mixture is stirred for 30-45 minutes then alkyl halide compound (0.65 mol) was added and the reaction mixture is heated to 55-65 °C and maintained for 2-3 hrs. The reaction mass was cooled to 25-35 °C , filtered and the filtrate was concentrated under vacuum. The residue obtained was dissolved in Toluene and the toluene layer was washed with water then concentrated under vacuum. (Note: For impurity II synthesis 1-bromo-4-chloro butane was used and for impurity III synthesis 1, 4-di bromo butane was used).

Impurity II: Yield: 90% Chemical purity by HPLC 97.01%, Impurity V- 0.56% and chiral purity is 99.58%ee; ^{1}H NMR ($CDCl_3$) δ 7.7 (1H, d, *J*=8.8Hz), 7.67 (1H, d, *J*=1.6Hz), 7.40 (1H, dd, *J*=1.6,8.6Hz), 7.14 (1H, m), 7.10 (1H, m), 4.11 (2H, m), 4.09 (2H,m), 3.89 (3H,s), 3.83(1H,q, *J*=7.6Hz), 1.65 (2H,m), 1.63 (2H,m), 1.58 (3H, d, *J*=6.8Hz); ^{13}C NMR (200 MHz) ($CDCl_3$); δ 174.4, 157.5, 135.3, 133.6, 129.1, 128.8, 127.0, 126.0, 125.8, 118.8, 105.5, 63.7, 55.2, 45.4, 44.3, 28.9, 25.9, 18.3; MS (DIP) m/*z* 321 (M^++1); IR (KBr, cm^{-2}): 1732.2 (-C=O), 1604 (-C=C), 1179.9,1169.2, 1027.9, 856.1, 822.5(Aromatic CH);

Impurity III: Yield: 85% Chemical purity by HPLC 96.01%, Impurity V- 0.6%; ^{1}H NMR ($CDCl_3$) δ 7.7 (1H, d, *J*=8.4Hz), 7.67 (1H, m), 7.39 (1H, dd, *J*=1.6,8.6Hz), 7.13 (1H, dd, *J*=2.4,8.8Hz) 7.10 (1H, m), 4.29 (2H, m), 4.09 (2H,m), 3.89 (3H,s), 3.83(1H,q, *J*=7.2Hz), 1.65 (2H,m), 1.63 (2H,m), 1.57 (3H, d, *J*=7.2Hz); ^{13}C NMR (200 MHz) ($CDCl_3$); δ 174.6, 157.4, 135.6, 133.5, 129.1, 128.7, 126.9, 126.0, 125.7, 118.8, 105.5, 64.5, 55.1, 45.4, 30.7, 28.8, 24.9, 18.3; MS (DIP) m/*z* 366 (M^++1); IR (KBr, cm^{-2}): 1730.9 (-C=O), 1606 (-C=C), 1177.1,1063.1, 858.4, 811.6 (Aromatic CH);

Synthesis of Impurity-IV:

(S)-Naproxen (10.0 gm, 0.04 mol), 1, 4-butanediol (39.13 gm, 0.4 mol) and Toluene 25.0 ml are charged into a round bottom flask and stirred for 5-10 min at ambient temperature, Sulfuric acid (0.21 gm, 0.002 mol) is added and the mixture is heated to 80°C and stirred for 8 hrs, The reaction mass was cooled to 25-35°C , reaction mass was washed with 10% sodium bicarbonate solution followed by 5% sodium chloride solution then the organic layer was concentrated under vacuum.

Yield: 83% Chemical purity by HPLC 94.01%,; ^{1}H NMR ($CDCl_3$) δ 7.7 (1H, d, *J*=8.8Hz), 7.65 (1H, m), 7.38 (1H, dd, *J*=1.6,8.6Hz), 7.13 (1H, dd, *J*=2.4,8.8Hz) 7.10 (1H, m), 4.1 (2H, t, *J*=7.2Hz), 3.89 (3H,s), 3.83(1H,q, *J*=7.2Hz), 3.52 (2H,t, *J*=6.8Hz), 1.65 (2H,m), 1.62 (2H,m), 1.57 (3H, d, *J*=7.6Hz); ^{13}C NMR (200 MHz) ($CDCl_3$); δ 174.4, 157.5, 135.5, 133.6, 129.1, 128.8, 127.0, 126.0, 125.7, 118.8, 105.5,64.0, 63.6, 55.2, 45.4, 27.1, 25.0, 18.3; MS (DIP) m/*z* 303 (M^++1); IR (KBr, cm^{-2}): 3359.6 (-OH), 1732.4 (-C=O), 1605.5 (-C=C), 1185.2,1055.9, 1028.1, 856.7, 823.4 (Aromatic CH);

Synthesis of Impurity-V:

(S)-Naproxen (10.0 gm) and acetonitrile (150 ml) are charged into a round bottom flask and stirred for 5-10 min at ambient temperature. K_2CO_3 (7.2 gm) is added and the mixture is stirred for 30-45 minutes then 1,4-di bromo butane (4.69 gm) was added and the reaction mixture is heated to 55-65 °C and maintained for 2-3 hrs. The reaction mass was cooled to 25-35 °C , filtered and the filtrate was concentrated under vacuum. The residue obtained was dissolved in Toluene and the toluene layer was washed with water then concentrated under vacuum.

Yield: 80% Chemical purity by HPLC 95.01%,; ^{1}H NMR ($CDCl_3$) δ 7.69 (2H, m), 7.65 (1H, m), 7.37 (2H, m), 7.13 (2H, m) 7.10 (2H, m), 4.0 (4H, m), 3.89 (6H,s), 3.8(2H,q, *J*=7.2Hz), 1.55 (4H,m), 1.50 (6H,m); ^{13}C NMR (200 MHz)

($CDCl_3$); δ 174.4, 157.5, 135.5, 133.5, 129.1, 128.8, 127.0, 126.1, 125.8, 118.8, 105.5,64.0, 55.2, 45.4, 25.0; MS (DIP) m/*z* 515 (M^++1); IR (KBr, cm^{-2}):1732.5 (-C=O), 1606.2 (-C=C), 1269.2, 2310.3, 1191.0, 1030.6, 854.7, 816.2 (Aromatic CH);

Synthesis of Naproxcinod:

Impurity II (2.4 Kg) and acetonitrile (4.8 L) are charged into a reactor and stirred at 25-35°C for 10 minutes. Silver nitrate (2.8 kg) is added and the mixture is heated to 80-85°C and stirred for 16-17 hrs, then cooled to 25-35°C. Reaction mass is diluted with acetonitrile (7.2 L) then filtered and concentrated. The crude compound was dissolved in dichloro methane then stirred for 1.0 hr at 0-5°C and filtered, filtrate was washed with water and the organic layer was distilled. Yield: 77.22%, Chemical purity: 98.9%, Impurity V- 0.496%; ^{1}H NMR ($CDCl_3$) δ 7.69 (1H, d, *J*=8.8Hz), 7.67 (1H, d, *J*=8.8Hz), 7.38 (1H, dd, *J*=1.6, 8.6Hz), 7.13 (1H, dd, *J*=2.4, 8.8Hz) 7.10 (1H, m), 4.29 (2H, t, *J*=6.4Hz), 4.09 (2H,m), 3.89 (3H,s), 3.83(1H,q, *J*=7.2Hz), 1.65 (2H,m), 1.63 (2H,m), 1.57(3H,d, *J*=7.2Hz); ^{13}C NMR (400 MHz) ($CDCl_3$); δ 173.9, 157.3, 135.3, 133.3, 128.7, 128.5, 126.7, 125.7, 125.4, 118.6, 105.1,72.2, 63.2, 54.6, 44.9, 24.3, 22.8, 17.8; MS (DIP) m/*z* 370 (M^++1); IR (KBr, cm^{-2}): 1731 (-C=O), 1627, 1280 (-NO_2), 1280,1031 (-C-O-C), 1177(-C-O), 856 (Aromatic CH);

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