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Short communication

Isolation and characterization of some potential impurities in ropinirole hydrochloride☆

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Abstract

Three impurities in ropinirole hydrochloride drug substance at levels ∼0.06–0.15% were detected by reverse-phase high performance liquid chromatography (HPLC). These impurities were isolated from the drug substance. These impurities were analyzed using reverse-phase HPLC. Based on the spectral data (IR, NMR and MS), structures of these impurities were characterized as 4-[2-(propylamino) ethyl]-1,3-dihydro-2*H*-indol-2-one hydrochloride (impurity-A), 5-[2-(diropylamino) ethyl]-1,4-dihydro-3*H*-benzoxazin-3-one hydrochloride (impurity-B) and 4-[2-(diropylamino) ethyl]-*1H*-indol-2,3-dione hydrochloride (impurity-C). Synthesis of these impurities is discussed.

Keywords: Ropinirole; Impurities; Isolation; Characterization

1. Introduction

Ropinirole [1], 4-[2-(dipropylamino) ethyl]-1,3-dihydro-*2H*-indol-2-one is a potent anti-parkinson's disease drug, which has been developed by SmithKline Beecham. There are few methods available [2–10] where authors reported liquid chromatography (LC)/liquid chromatography–mass spectrometry (LC–MS)/capillary zone electrophoresis (CZE)/capillary liquid chromatography (CLC) methods for quantification of ropinirole and its impurities. Some of the methods describe determination of ropinirole and its impurities in human, rat, drug plasma and evaluation of solid-phase sorbents, etc., while the others emphasize more on effect of purity of di-*N*-*n*-propyl amine, eigen value plots, peak purity determination, etc.

During the analysis of different laboratory batches of ropinirole hydrochloride, four impurities were detected consistently in almost all batches, whose area percentage ranged from ∼0.06 to ∼0.15% by reverse-phase HPLC method. One impurity was identified as a precursor of ropinirole hydrochloride and confirmed by spiking later. A comprehensive study has been taken to isolate and characterize the remaining three impurities by preparative HPLC and spectroscopic techniques. The impurity profile study has to be carried out for any final drug substances to identify and characterize all unknown impurities that are present at a level above 0.05% [11]. The requirement of identification and characterization of these impurities in the final drug substances is extremely necessary to meet the stringent regulatory requirements. A preparative LC method has been developed to isolate these impurities from the drug substances. An inhouse reverse-phase analytical HPLC method has been developed and validated further to analyse these impurities. The paper not only describes isolation and characterization of these impurities but also explains probable path for formation of these impurities in bulk drug substance. Synthesis of these impurities is also discussed.

Abbreviations: LC, liquid chromatography; HPLC, high performance liquid chromatography; LC–MS, liquid chromatography–mass spectrometry; CZE, capillary zone electrophoresis; CLC, capillary liquid chromatography; GR, guaranteed reagent; AR, analytical grade; PDA, photo diode array; UV, ultra violet; FT-IR, Fourier transform infra red; ESI, electron spray ionization; DP, declustring potential; EP, entrance potential; NMR, nuclear magnetic resonance; APT, attached proton test; RF, retention factor; DMSO, dimethyl sulfoxide; D_2O, deuterium oxide

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2. Experimental

2.1. Chemicals and samples

The investigated samples of ropinirole hydrochloride drug substances were obtained from process research development laboratory of BioArc Research Solutions, Alembic limited, Vadodara, Gujrat, India. HPLC grade acetonitrile and methanol were obtained from Spectrochem (India), potassium dihydrogen phosphate (GR grade), *ortho*-phosphoric acid (GR grade) and triethyl amine (Synthesis) were used from Merck (India). Ammonium aceteate (AR grade) was used from Rankem (India). Water used for preparing mobile phase was obtained from milli-Q gradient water purification system.

2.2. Apparatus

A Shimadzu analytical LC-2010 HT separation module equipped with UV detector was used. Data was recorded and processed using LC solution software. A Shimadzu preparative HPLC equipped with LC-8A pump, SCL-8A system controller, SPD-M10 Avp photodiode array (PDA) detector and rheodyne injector model 7725i with 10 ml loop was used for isolating impurities. Virtis 4 l lyophilizer and a buchi rotavapor R-200 were used.

2.3. High performance liquid chromatography (HPLC) (analytical)

The analysis was carried out on a Symmetry C_8 column having dimensions 250 mm × 4.6 mm i.d. and 5 μm particle size column using a mobile phase A: potassium dihydrogen phosphate (0.035 M, pH 6.0 adjusted with *ortho*-phosphoric acid)–triethyl amine–acetonitrile (90:0.5:10, v/v/v); mobile phase B: acetonitrile–water–triethyl amine (80:20:0.2, v/v/v) (pH 6.0 adjusted with *ortho*-phosphoric acid) with a timed gradient programme of time (T_{min})/%B: 0/5, 20/5, 37/60, 47/60, 50/5, 60/5 and a flow rate of 1.0 ml/min, UV detection at 250 nm was used. The column was maintained at 45 °C throughout the analysis. The diluent was mobile phase A–mobile phase B (95:5, v/v).

2.4. High performance liquid chromatography (preparative)

The isolation of impurities was carried out on a Inertsil C_{18} column having dimensions 250 mm × 20 mm i.d. and 8 μm particle size using a mobile phase A: ammonium acetate (0.05 M); mobile phase B: mixture of acetonitrile–methanol (50:50, v/v) with a timed gradient programme of time (T_{min})/%B: 0/25, 5/25, 10/35, 15/50, 25/70, 30/80, 40/80, 45/25, 55/25 and a flow rate of 15.0 ml/min, UV detection at 250 nm was used. About 100 mg of drug substance was used to isolate impurities-A and -B and about 25 mg of the drug substance was used to isolate impurity-C. The diluent was water–methanol (70:30, v/v).

Desalting and further purification of the isolated compound of about 90% purity was carried out on a Inertsil C_{18} column having dimensions 250 mm × 20 mm i.d. and 8 μm particle size using a mobile phase A: milli-Q water; mobile phase B: mixture of acetonitrile–methanol (50:50, v/v) with a timed gradient programme of time (T_{min})/%B: 0/0, 5/0, 10/40, 15/50, 25/70, 30/80, 40/80, 45/25, 50/0, 60/0 and a flow rate of 15.0 ml/min, UV detection at 250 nm was used. About 10 ml of the sample was loaded onto column. The diluent was water–methanol (70:30, v/v).

2.5. Mass spectrometry

ESI mass spectra were obtained using AB Sciex API-2000 mass spectrometer in positive mode with DP and EP 20 and 10 V, respectively. The samples dissolved in methanol were introduced using Perkin-Elmer pump and an injector.

2.6. NMR spectroscopy

NMR measurements (^1H and ^{13}C) were performed on a Bruker Avance 300 MHz instrument at 25 °C.

2.7. FT-IR spectroscopy

IR spectra were recorded in the solid state as KBr dispersion using Perkin-Elmer, spectrum-one FT-IR spectrophotometer.

2.8. Isolation of impurities by preparative HPLC

A reverse-phase solvent system discussed under Section 2.3 was used for the isolation of impurities. The sample was loaded on the preparative column, fractions collected, analyzed by liquid chromatography to confirm the RF of the isolated fractions and were pooled together and kept in a refrigerator. The pooled fractions were concentrated under high vacuum buchi rotavapor to strip off the organic solvent. The remaining aqueous layer was subjected to lyophilization in vertis 4 l lyophilizer to get a compound. The compound obtained was again loaded on preperative column for its purification and desalting. The fraction thus collected was subjected to organic solvent evaporation and lyophilization to get the purified compound. The chromato-

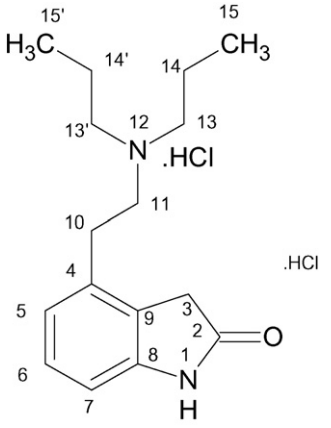


Fig. 1. chemical structure of ropinirole hydrochloride.

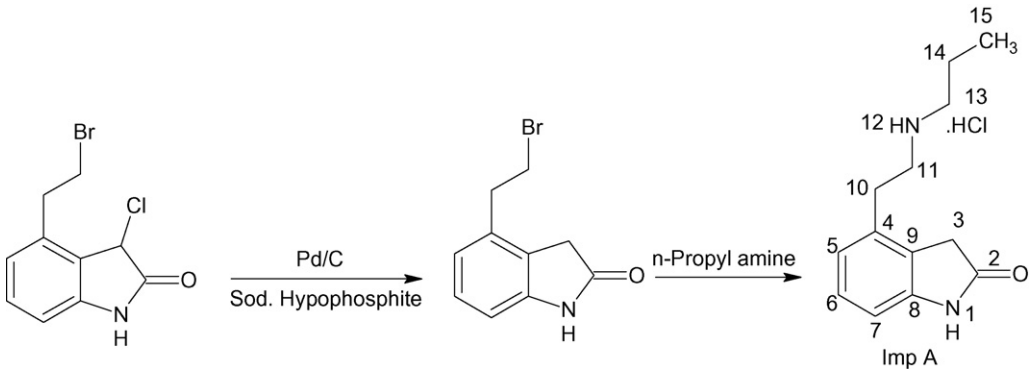


Fig. 2. Scheme for synthesis of impurity-A.

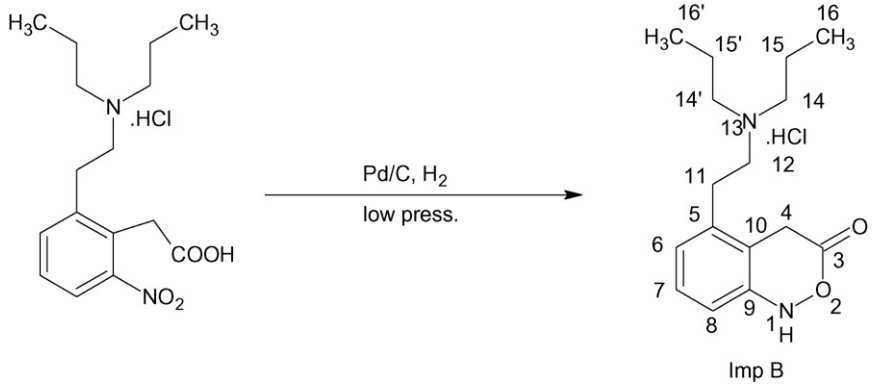


Fig. 3. Scheme for synthesis of impurity-B.

graphic purity of the impurities were tested by analytical LC and found to be 93.18, 97.68 and 95.03%, respectively. The isolated impurities were used to generate spectral data.

2.9. Synthesis of impurities

Impurity-A was synthesized by the alkylamination of 4-bromoethylindol-2-one. Impurity-B was synthesized by partial reduction of {2-nitro-6-[2-(propylamino) ethyl] phenyl} acetic acid hydrochloride. Impurity-C was prepared by oxidation of ropinirole hydrochloride using SeO_2.

The reaction masses obtained after the synthesis were subjected to LC using the method discussed in Section 2.2. Interestingly the percentage purities of the impurities were found to be 95.21, 98.22 and 97.18%, respectively. The spectral data of the synthesized impurities matched well with those isolated from preparative HPLC. This was further confirmed by spiking the isolated and synthesized impurities in the sample and analyzing it by LC. Structure of ropinirole hydrochloride and proposed synthetic schemes are depicted in Figs. 1–4.

(1) Proposed synthetic scheme for impurity-A (see Fig. 2).
(2) Proposed synthetic scheme for impurity-B (see Fig. 3).
(3) Proposed synthetic scheme for impurity-C (see Fig. 4).

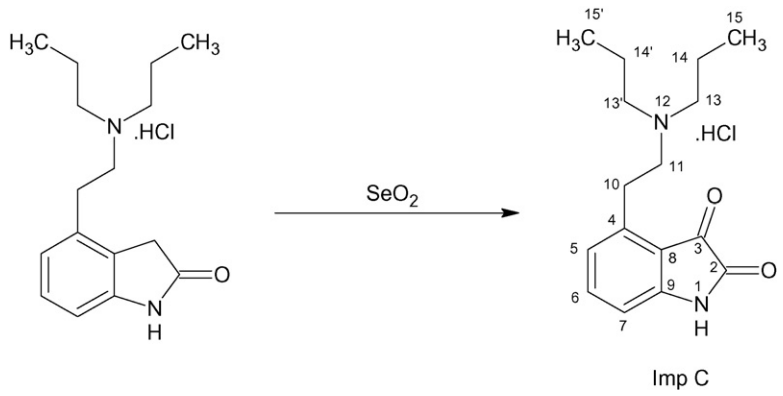


Fig. 4. Scheme for synthesis of impurity-C.

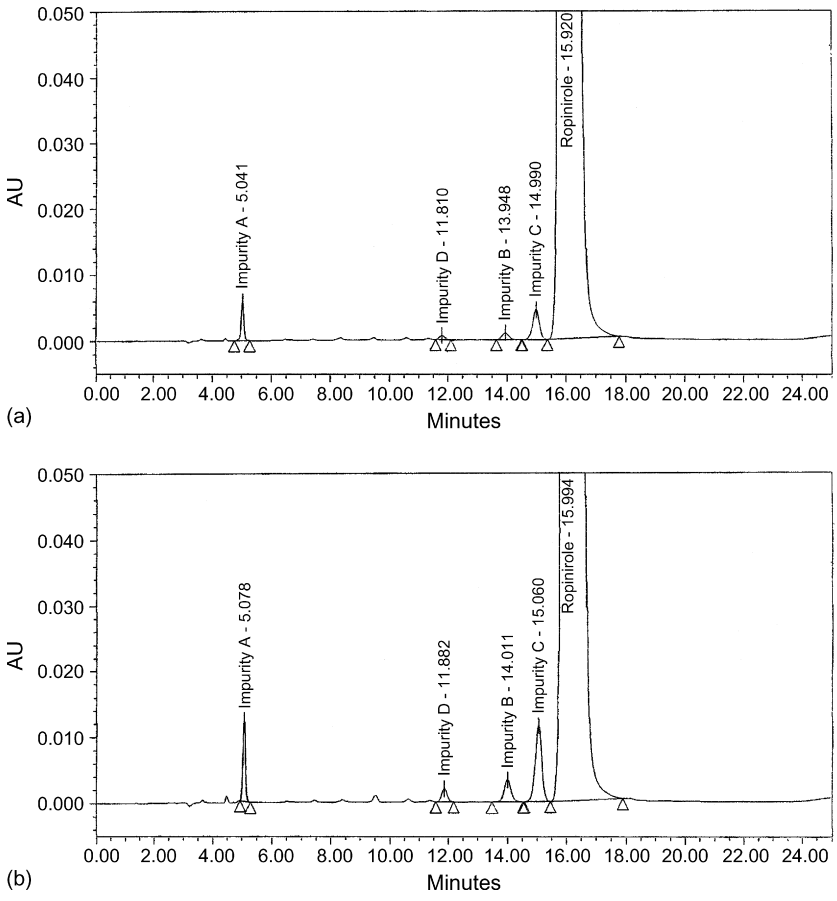


Fig. 5. (a) HPLC chromatogram of ropinirole drug substance. (b) HPLC chromatogram of ropinirole drug substance spiked with isolated impurities.

3. Results and discussion

3.1. Detection of impurities

A typical analytical LC chromatogram (Fig. 5a) of a laboratory ropinirole hydrochloride bulk drug as is and spiked with isolated impurities (Fig. 5b) recorded using LC method as described in Section 2.2. The target impurities under study are marked as imp-A, imp-B, imp-C. Impurity-D was identified as {2-nitro-6-[2-(propylamino) ethyl] phenyl} acetic acid *N*-oxide, which is a precursor of ropinirole hydrochloride. Retention factor (RF), mass obtained as $M + H^+$, nature and structures of these impurities and ropinirole hydrochloride are shown in Table 1. Impurities were isolated from ropinirole hydrochloride drug substance on preparative chromatography. Attempts were also made later to synthesize these impurities.

3.2. Structure elucidation of impurities

3.2.1. Structure elucidation of impurity-A

The ESI mass spectrum of impurity-A exhibited a molecular ion peak at *m/z* 219.4 atomic mass unit (amu) which was less in 42 amu than ropinirole. In the FT-IR spectrum a characteristic absorption band is appeared at 1695 cm^{-1} for >C=O stretching and at 3436 cm^{-1} for >N–H stretching vibration. Aliphatic asymmetric and symmetric >C–H stretching appeared in the range 2962–2858 cm^{-1}. Aromatic >C–N stretching observed at 1325 cm^{-1}. In the ^1H spectrum of the impurity-A in dimethyl sulfoxide (DMSO) 13 protons appeared in the up field region (0.88–3.50 ppm), 3 in the downfield region (6.70–7.15). Deuterium oxide (D_2O) exchange showed presence of –NH proton in the compound. ^{13}C attched proton test (APT) shows presence of a $–CH_3$ groups at 11.94 ppm, a >C=O at 177.00 ppm. A detailed description is given in the Tables 2 and 3.

Based on the above spectral data the molecular formula of impurity-A was confirmed and the corresponding structure was characterized as 4-[2-(propylamino) ethyl]-1,3-dihydro-*2H*-indol-2-one hydrochloride (Table 4).

3.2.2. Structure elucidation of impurity-B

The ESI mass spectrum of impurity-B exhibited a molecular ion peak at *m/z* 277.1 amu, which was high in 16 amu than ropinirole. In the FT-IR spectrum, a characteristic absorption band is appeared at 1727 cm^{-1} for >C=O stretching and at 3432 cm^{-1} for >N–H stretching vibration. Aliphatic asymmetric and symmetric >C–H stretching appeared in the range 2962–2819 cm^{-1}. Aromatic >C–N stretching observed at 1320 cm^{-1}. In the ^1H spectrum of the Impurity-B, in DMSO 20 protons appeared in the up field region (0.88–3.63 ppm), 3 in the downfield region (6.79–7.26) and a –NH prton appeared at 10.78 ppm. D_2O

Table 1
Details of retention factor, mass and nature of ropinirole hydrochloride, impurity-A, impurity-B and impurity-C

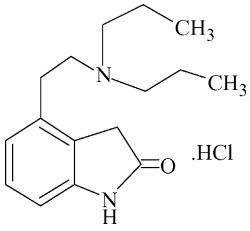
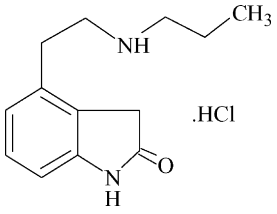
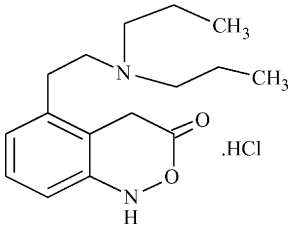
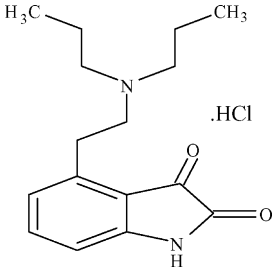
S. no.	Compound	Analytical RF	Structure	MS analysis $(M+H)^+$	Nature
01	Ropinirole	1.0		261.5	Drug substance
02	Impurity-A	0.32		219.4	Process related
03	Impurity-B	0.88		277.1	Process related
04	Impurity-C	0.93		275.5	Process related

Table 2
Comparative ^{1}H NMR assignments of ropinirole hydrochloride, impurity-A, impurity-B and impurity-C

A	Ropinirole			Impurity-A			Impurity-B			Impurity-C		
Pos^n	1H	δ (ppm)	J (Hz)	1H	δ (ppm)	J (Hz)	1H	δ (ppm)	J (Hz)	1H	δ (ppm)	J (Hz)
1	1	10.45	s	1	10.47	s	1	10.78	s	1	10.7	bs
3	2	3.53	s	2	3.505	s						
4							2	3.63	s			
5	1	6.7–6.72	d, 7.73	1	6.70–6.73	d, 7.5				1	6.96–6.98	d, 7.65
6	1	7.08–7.13	t, 15.37	1	7.09–7.15	t, 15.6	1	6.92–6.95	d, 7.68	1	7.47–7.52	t, 15.57
7	1	6.82–6.84	d, 7.65	1	6.79–6.81	d, 7.5	1	7.21–7.26	t, 15.33	1	6.8–6.82	d, 6.45
8							1	6.80–6.82	d, 7.56			
10	2	3.14–3.19	m	2	2.82	b				2	3.19–3.28	m
11							2	3.18–3.2	b	2	3.11–3.15	m
11				2	2.93	m						
11+13+13′	6	2.94–3.04	m							4	3.01–3.08	m
12+14+14′							6	3.048	m			
13				2	3.018	b						
14				2	1.62–1.70	q						
14+14′	4	1.63–1.75	m							4	1.77–1.66	m
15				3	0.88–0.92	t, 12.00						
15+15′	6	0.87–0.91	t,14.43				4	1.68–1.75	q	6	0.89–0.93	t, 19.8
16+16′							6	0.88–0.93	t, 14.16			

d, doublet; s, singlet; t, triplet; m, multiplet; bs, broad singlet; brm, broad multiplet; b, broad; J, coupling constant.

Table 3
Comparative ^{13}C NMR APT assignments of ropinirole hydrochloride, impurity-A, impurity-B and impurity-C

A	Ropinirole		Impurity-A		Impurity-B		Impurity-C	
Pos[n]	δ (ppm)	APT	δ (ppm)	APT	δ (ppm)	APT	δ (ppm)	APT
2	176.99	C=O	177	C=O			159.91	C=O
3	35.49	CH_2	29.78	CH_2	170.03	C=O	185.47	C=O
4	133.94	Ar–Cq	125.78	Ar–Cq	27.07	CH_2	152.05	Ar–Cq
5	108.63	Ar–C–H	108.58	Ar–C–H	128.87	Ar–C–H	125.15	Ar–C–H
6	128.66	Ar–C–H	128.63	Ar–C–H	121.04	Ar–Cq	139.14	Ar–C–H
7	122.53	Ar–C–H	122.28	Ar–C–H	123.39	Ar–C–H	111.85	Ar–C–H
8	144.64	Ar–Cq	144.61	Ar–Cq	106.49	Ar–C–H	116.73	Ar–Cq
9	125.9	Ar–Cq	134.23	Ar–Cq	144.66	Ar–Cq	138.86	Ar–Cq
10	27.69	CH_2	35.56	CH_2	133.87	Ar–Cq	25.93	CH_2
11	52.50	CH_2	47.24	CH_2	33.27	CH_2	51.80	CH_2
12					52.54	CH_2		
13			49.08	CH_2				
13+13′	53.9	CH_2					54.34	CH_2
14			19.77	CH_2				
14+14′	17.23	CH_2			53.96	CH_2	17.37	CH_2
15			11.94	CH_3				
15+15′	11.81	CH_3			17.26	CH_2	11.80	CH_3
16								
16+16′					11.81	CH_3		

Ar–Cq, aromatic quaternary carbon; Ar–C–H; aromatic C–H.

Table 4
FT-IR frequency assignments of ropinirole, impurity-A, impurity-B and impurity-C all frequencies are in cm^{-1}

Mode of vibration	Ropinirole	Impurity-A	Impurity-B	Impurity-C
>C=O stretching	1703	1695	1727	1748, 1721
>N–H stretching	3416	3436	3432	3438
Aliphatic asymmetric and symmetric >C–H	2881–2970	2962–2858	2962–2819	2958–2808
>C–N stretching	1324	1325	1320	1318

exchange showed presence of –NH proton in the compound. ^{13}C APT shows presence of 2 $–CH_3$ groups at 11.81 ppm, a >C=O at 170.03 ppm. A detailed description is given in the Tables 2 and 3.

Based on the above spectral data the molecular formula of impurity-B was confirmed and the corresponding structure was characterized as 5-[2-(dipropylamino) ethyl]-1,4-dihydro-3*H*-benzoxazin-3-one hydrochloride.

3.2.3. Structure elucidation of impurity-C

The ESI mass spectrum of impurity-C exhibited a molecular ion peak at *m/z* 275.5 amu which was high in 14 amu than ropinirole. In the FT-IR spectrum, a characteristic absorption bands appeared at 1741 and 1728 cm^{-1} for >C=O stretching and at 3438 cm^{-1} for >N–H stretching vibration. Aliphatic asymmetric and symmetric >C–H stretching appeared in the range 2958–2808 cm^{-1}. Aromatic >C–N stretching observed at 1318 cm^{-1}. In the ^1H spectrum of the impurity-C in DMSO 18 protons counted in the up field region (0.87–3.28 ppm), 3 in the downfield region (6.8–7.52) and a –NH proton at 10.7 ppm. D_2O exchange showed presence of –NH proton in the compound. ^{13}C APT shows presence of 2 $–CH_3$ groups at 11.8 ppm, a >C=O at 159.91 and 185.47 ppm. A detailed description is given in the Tables 2 and 3.

Based on the above spectral data the molecular formula of impurity-C was confirmed and the corresponding structure was characterized as 4-[2-(dipropylamino) ethyl]-*1H*-indol-2,3-dione hydrochloride.

3.3. Formation of impurities

Hydrogenation of {2-nitro-6-[2-(propylamino) ethyl] phenyl} acetic acid hydrochloride present in the precursor of ropinirole hydrochloride is responsible for the formation of impurity-A. Impurity-B is formed due to partial reductive cyclization of {2-nitro-6-[2-(propylamino) ethyl] phenyl} acetic acid hydrochloride. Impurity-C could be formed due to arial oxidation of ropinirole hydrochloride in solution.

4. Conclusion

The present study illustrates isolation of three process related unknown impurities of ropinirole hydrochloride by preparative HPLC which were further characterized using various spectroscopic techniques.

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