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Isolation And Structure Elucidation Of A New Impurity Of Ropinirole Hydrochloride In Solid Dosage Form

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Abstract: A new unknown impurity of Ropinirole hydrochloride (ROP) in tablet formulation was found during HPLC analysis of stability samples of ROP tablets. The structure of this impurity was elucidated as "3, 3'-methanediylbis {4-[2-(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one}". The impurity was enriched, isolated using preprative HPLC and its structure was confirmed by comparision with the chromatographic retention characteristic using ultra-violet wavlength detection, nuclear magnetic rasonance spectroscopy (NMR) and mass spectroscopy (MS). The presence of 3, 3'-methanediylbis {4-[2-(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one} in ROP tablets has not been described previously.
Keywords: Ropinirole, lactose, isolation, stability.

1.0 Introduction

Stability testing is used as a primary tool to determine and asses the expiration dating and storage conditions for pharmaceutical products. Stability testing includes long-term and accelerated conditions, where the product is stored at room temperature and humidity, high heat and controlled humidity conditions. Stability study results are the evidence of establishment and assurance of safety, quality and efficacy of the drug product. In order to determine the product stability, the appropriate physical, chemical, biological and microbiological testing must be performed. One of the evaluation criteria is the appearance of impurities during real time and accelerated stability studies.

ROP, chemically known as 4-[2-(Dipropylamino)ethyl}-1,3-dihydro-2(H)-indol-2-one monohydrochloride is nonergot-derivative dopamine receptor agonist and is used as an symptomatic management of idiopathic parkinsonian syndrome [1,2] and restless legs syndrome [3].

Literature survey reveals some analytical methods [4, 8] by LC/MS, by liquid chromatography, and by capillary zone electrophoresis (CZE) for estimation of ropinirole in pharmaceutical dosage form and in biological fluids. A stability indicating related substance method is reported for estimation of ropinirole and its impurities by UPLC [9]. The isolation of three process related unknown impurities of ropinirole hydrochloride by preparative HPLC which were further characterized using various spectroscopic techniques is also reported by B. Sahastrabuddhey and et al. [10].

Besides several known and unknown impurities, one additional compound at level >0.2% was detected by HPLC. As per the stringent regulatory requirements recommended by ICH, the impurities >0.2% must be identified and characterized.

This paper elaborates an unknown impurity of ROP which was formed in stability samples. Chromatograph of stability sample of ROP Tablets is shown in Fig.1. The unknown impurity is eluated at relative retation time

about 2.05 and level was out of limit as per ICH guidelines. Therefore, it was necessary not only to meet the stringent regulatory requirement, but also to get an insight into the possible route of formation of the impurity. This impurity further investigated for route of formation, each excipient was check for possible formation with interaction and final it was observed ROP tablets contains lactose which in basic pH, releases formaldehyde, and ROP have an active methylene group to react with formaldehyde to form methylene bridge dimmer impurity by aldol condensation reaction

The unkown impurity in ROP tablets was issolated by preparative HPLC and characterized by spectral analysis.

2.0 Experimental

2.1 Materials and reagents

Reference standards, bulk drug of ROP, Lactose and ROP tablets were provided by Torrent pharmaceuticals ltd. Sodium hydroxise (analytical grade), Hydrochloric acid (Analytical grade) Ammonia solution (analytical grade), acetonitrile (HPLC grade), methanol (HPLC grade), methylene chloride (analytical grade) was procured from Merck India Limited (Mumbai, India) and water was purified with a Milli-Q plus system from Millipore were used.

2.2 Chromatographic system (analytical HPLC)

A Shimadzu model LC-2010C (Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan) chromatograph with auto injector, sample cooler, and UV–Visible and Photodiode array (PDA) detector (SPD 10mA vp), connected to data processing system software (Class-VP 6.13 SP2) was employed. In the developed method, Hypersil-BDS C8 (4.6X150 mm, 5 µ), was maintained at ambient. Separation of ROP and its unknown degradation product was achieved under isocratic conditions using mixture of buffer (pH 2.5, 0.05M potassium dihydrogen phosphate), acetonitrile and methanol (85:10:5, v/v) as mobile phase. The monitoring wavelength was 250 nm and the flow rate was 1.2 ml/min.

2.3 Preparation of impurity

ROP API and lactose were mixed in 1:3 ratio (w/w) in 200 ml of water and pH was adjusted to 12 by 1N sodium hydroxide solution. This solution was stirred for 4 hours at 40°C temperature. This solution was neutralized with diluted hydrochloric acid and injected on HPLC and observed that the impurity with RRT of 2.05 was enriched to 20 %.

2.4 Preparative HPLC

Waters preparative HPLC (Delta Prep) 4000 system (Waters Corporation, Milford, MA, USA) with high pressure unit of 4000 psi was used. It was operated through Empower software. XTerra (Waters) C18 column (30X100 mm), preparative column packed with 5 µ particle size was employed for separation and isolation of the impurity. The mobile phase A was prepared by adjusting mili-Q water pH 10.5 with dilute ammonia, while mobile phase B was methanol and mobile phase C was acetonitrile. The gradient programme used was time/%B/%C: 0/0/0, 1.5/0/0, 2/60/20, 9/70/20, 9.01/ 100/0, 12/100/0,12.01/0/0 and 14/0/0. Flow rate was kept at 45ml/min and UV detection was at 250 nm.

2.5 Isolation of impurity by preparative HPLC

The impurity enriched sample was loaded on the preparative column using chromatographic conditions mentioned under preparative HPLC. The isolated fractions were collected and analyzed by analytical HPLC using conditions HPLC chromatographic conditions. These fractions were collected and pooled together. The collected fraction was having purity greater than 99.0%. The solvents of fraction were evaporated using rotavapor. This impurity was spiked in ROP tablet sample and analyzed on HPLC. The respective chromatograph is shown in Figure 2. The retention time of an unknown impurity in tablet and in spiked sample was comparable.

2.6 Mass spectrometry

The mass spectra of an unknown impurity were recorded on Thermofinnigan LXQ. Detection of ions was performed in Electrospray ionization, positive ion mode. The mass spectrum of unknown impurity shows mass of 533.3(M+1).

2.7 NMR (Nuclear magnetic resonance) spectrometry

The ^{1}H NMR, ^{13}C, DEPT-135, COSY and HSQC NMR experiments were performed on AVANCE DPX 400 Bruker NMR spectrophotometer in DMSO-d6.

Figure-1: Chromatograph of stability sample of ROP tablet

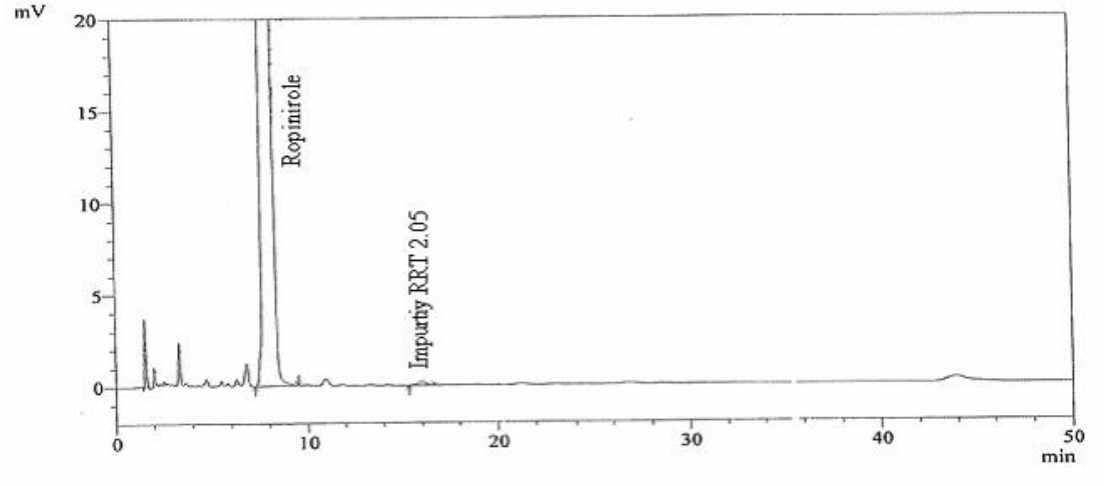
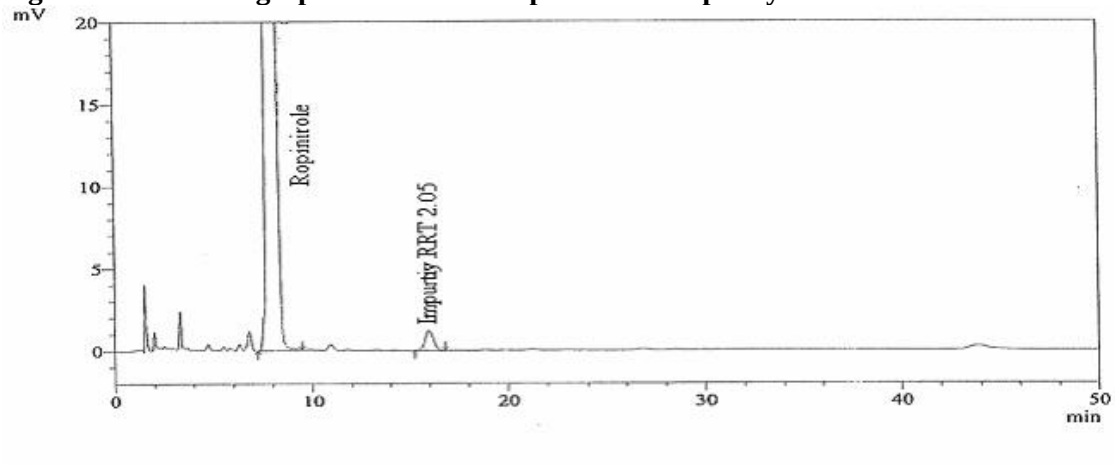


Figure-2: Chromatograph of ROP tablet spiked with impurity RRT 2.05.



3.0 Results And Discussion

3.1 Isolation of Impurity of ROP

ROP and lactose were subjected to stress condition to generate impurity, which was detected at RRT of 2.05 with respect to ROP. This degraded solution was subjected to preparative chromatography (section 2.4) to enable the isolation of the impurity. The purity of this solid was 95.16%. The impurity was then characterized by NMR (^{1}H and ^{13}C) and mass spectrometry.

3.2 NMR and Mass characterization of ROP.

For structure elucidation of unknown degraded impurity, characterization of ROP API was done by NMR and Mass then compared it with NMR and Mass spectra of an unknown degraded impurity.

The Electrospray ionization (ESI) mass spectrum of ROP API shows a molecular ion peak at m/z of 261 [M+1].^{1}H and ^{13}C NMR of ROP API performed in DMSO-D6 solvent.

NMR characterization data of ROP is shown in Table 1.

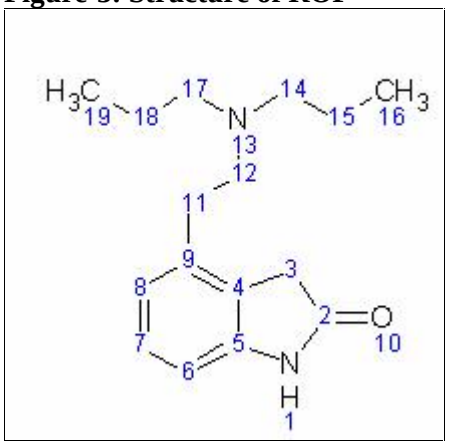
The structure of ROP is elucidated as 4-[2-(Dipropylamino)ethyl}-1,3-dihydro-2(H)-indol-2-one monohydro chloride. It is shown in Figure 3.

Table1: NMR Characterization of Ropinirole

Position	1H Chemical shift δ ppm	Multiplicity	^{13}C Chemical Shift	Proton /Carbon assignment
1	10.56	BS[e]	-	NH
2	-	-	176.64	C=O
3	3.55	S[a]	35.10	CH_2
4	-	-	133.57	C
5	-	-	144.24	C
6	6.73	D[c]	108.24	CH
7	7.14	T[b]	128.27	CH
8	6.86	D[c]	122.16	CH
9	-	-	125.53	C
10	-	-	-	-
11	3.19	M[d]	27.27	CH_2
12	3.05	M[d]	52.05	CH_2
13	-	-	-	-
14	3.05	M[d]	53.38	CH_2
15	1.70	M[d]	16.81	CH_2
16	0.92	T[b]	11.43	CH_3
17	3.05	M[d]	53.38	CH_2
18	1.70	M[d]	16.81	CH_2
19	0.92	T[b]	11.43	CH_3

[a]S Singlet. [b]T Triplet. [c]D Doublet. [d]M Multiplet. [e]BS Broad Singlet

Figure-3: Structure of ROP



3.3 Structure elucidation of an unknown degraded impurity RRT 2.05

The Electrospray ionization (ESI) mass spectrum of an impurity with RRT 2.05, shows a molecular ion peak at m/z of 533.3 [M+1] while ROP mass exhibited at 261 (m+1). Moreover MS-MS of 533 ion give 261 and 273 ion fragmentation in which 261 mass corresponding to ROP while 273 corresponding to an unknown moiety.

In ^{1}H NMR study of an impurity with RRT 2.05, spectrum shows two extra signals observed at 2.14 δ ppm and 4.44 δ ppm. 2D-Cosy spectra of impurity with RRT 2.05 shows 2.14 δ ppm chemical shift correlate to 4.44 ppm. Means both protons are adjutant proton. In DEPT-135 NMR spectra of impurity with RRT 2.05, one signal at 35 ppm (3-CH2 in ROP) was disappear and two additional signals observed at 40.3 ppm corresponding to –CH signal and 32.15 ppm corresponding to –CH2. 2D-HSQC spectra of impurity with RRT 2.05 shows 2.14 δ ppm protons correlate to 32.15 δ ppm carbon which confirms–CH2 by Dept-135 and 4.44 ppm proton correlated to 40.3 ppm carbon which confirms –CH by DEPT-135 experiment. Spectrum of ^{1}H NMR, 13C

NMR spectra, Cosy NMR spectra, DEPT-135 NMR spectra and HSQC NMR spectra are shown in fig. 4 (A), (B), (C), (D) and (E) respectively.

NMR characterization data of impurity with RRT 2.05 are shown in Table 2.

Figure-4 (A): ^{1}H NMR spectra of an impurity with RRT 2.05

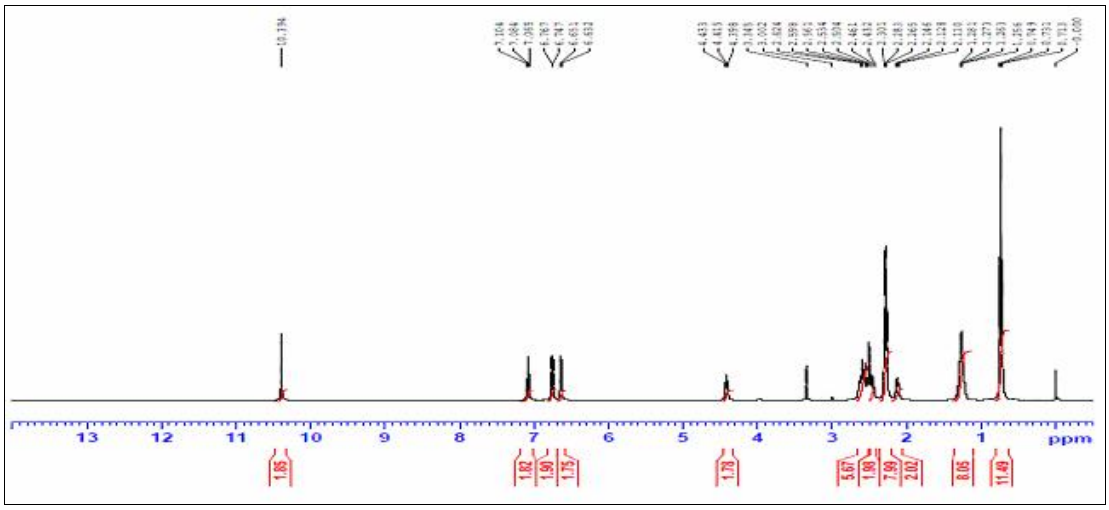


Figure-4 (B): ^{13}C NMR spectra of an impurity with RRT 2.05

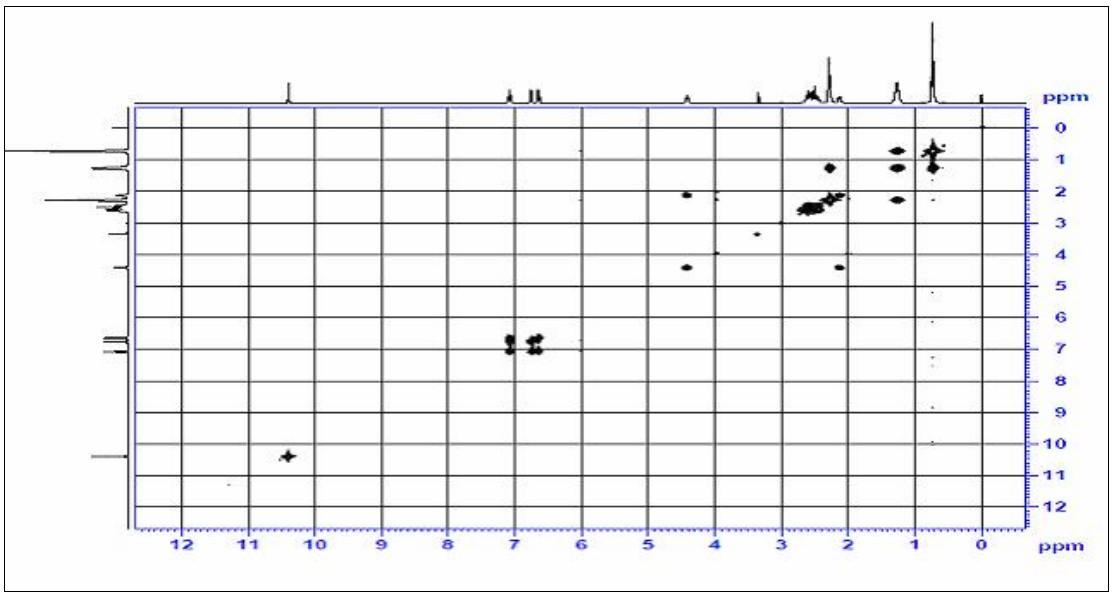


Figure-4 (C): COSY NMR spectra of an impurity with RRT 2.05

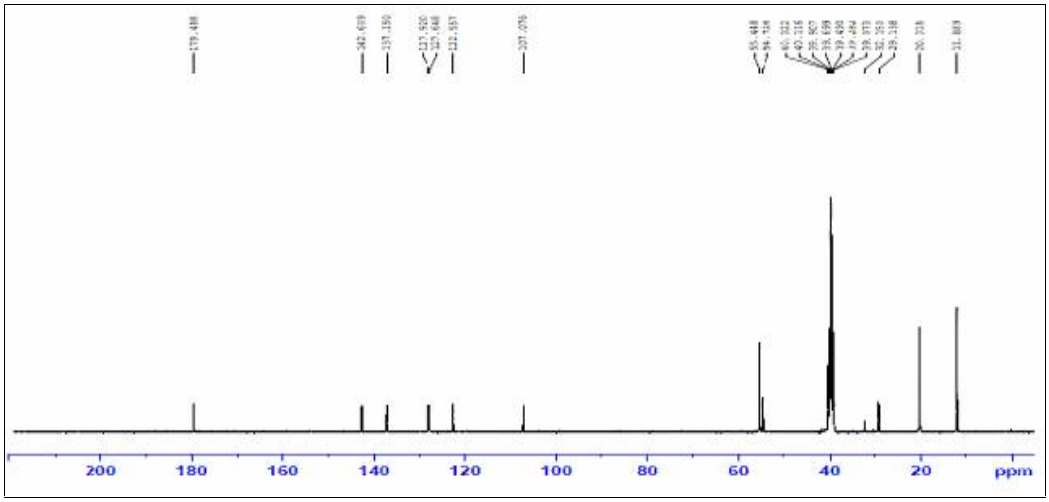


Figure-4 (D): DEPT-135 NMR spectra of an impurity with RRT 2.05

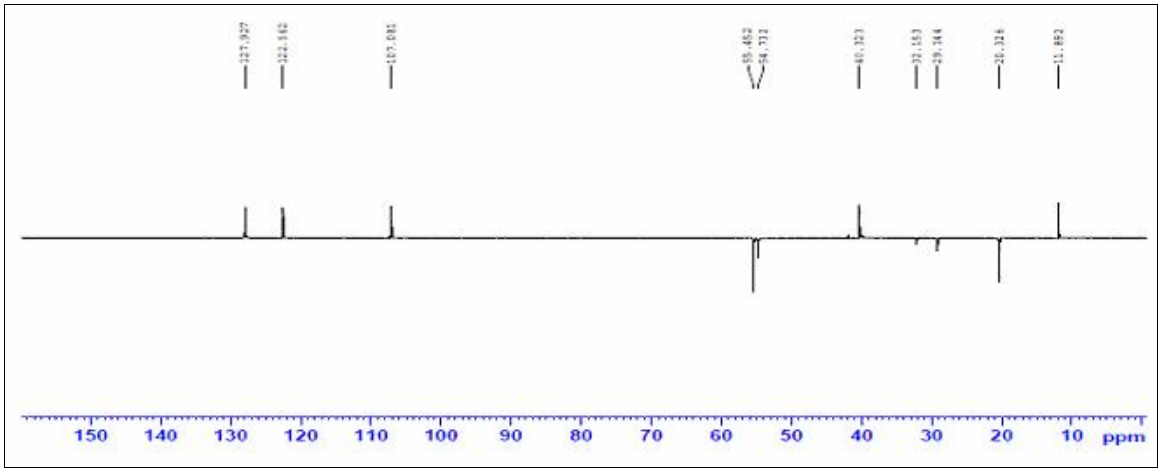
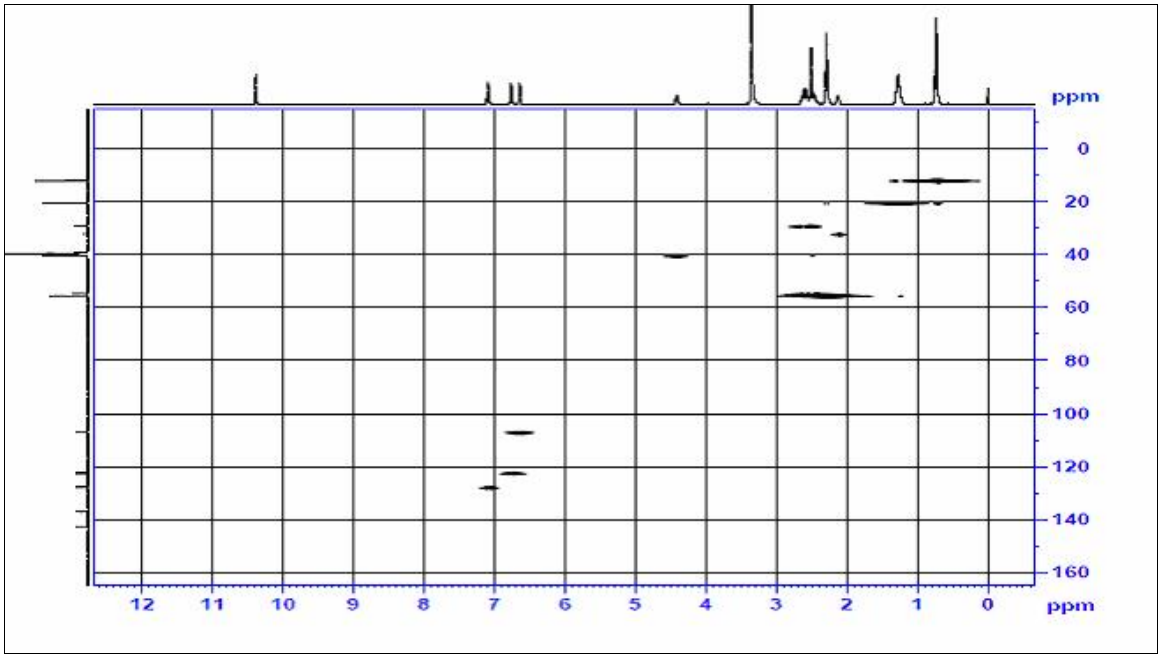


Figure-4 (E): HSQC NMR spectra of an impurity with RRT 2.05



The structure of an unknown degraded impurity RRT 2.05 is elucidated as 3,3'-methanediylbis {4-[2-(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one}. It is shown in Figure 5.

3.4 Mass Fragmentation of 2.05 RRT

Mass fragmentation of an unknown degraded impurity RRT 2.05 is shown in Fig. 6. MS spectra and MS-MS spectra of impurity with RRT 2.05 are shown in figure 7(A) and 7(B) respectively.

4.0 Conclusions

Determining the structures of impurity arising in the course of stress testing can be useful for preclinical discovery efforts during structure–activity relationship investigations. ROP reacts with lactose in presence of water at basic pH to form methylene bridge dimmer impurity molecules, which was detected by HPLC and isolation of impurity was achieved using preparative chromatography. The structure of this impurity was characterized by IR, NMR (^{1}H and ^{13}C) and mass spectroscopic studies and elucidated as 3,3'-methanediylbis{4-[2-(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one}.

Table-2: NMR Characterization of Ropinirole 2.05 RRT impurity

Position	^{1}H Chemical shift δ ppm	Multiplicity	^{13}C Chemical Shift	DEPT -135	COSY ^{1}H-^{1}H	HSQC ^{1}H-^{13}C	Proton /Carbon Assignment
1,14	10.39	S[a]	-	-	-	-	NH
2,13	-	-	179.49	-	-	-	C=O
3,12	4.41	T[b]	40.32	CH	2.12	4.41-40.32	CH
4,16	-	-	137.15	-	-	-	C
5,15	-	-	142.61	-	-	-	C
6,20	6.63	D[c]	107.07	CH	7.08	6.63-107.07	CH
7,19	7.08	T[b]	127.92	CH	6.63,6.74	7.08-127.92	CH
8,18	6.74	D[c]	122.56	CH	7.08	6.74-122.56	CH
9,17	-	-	127.65	-	-	-	C
11	2.12	T[b]	32.15	CH_2	4.41	2.12-32.15	CH_2
21,28	2.50-2.62	M[d]	29.14	CH_2	-	(2.50-2.62) -29.14	CH_2
22,29	2.50-2.62	M[d]	54.72	CH_2	-	(2.50-2.62) -54.72	CH_2
24,37, 31,34	2.28	T[b]	55.45	CH_2	1.25-1.28	2.28-55.45	CH_2
25,38, 32,35	1.25-1.28	M[d]	20.32	CH_2	2.28,0.71	(1.25-1.28)-20.32	CH_2
26,33, 36,39	0.71	T[b]	11.89	CH_3	1.25-1.28	0.71-11.89	CH_3

[a]S Singlet. [b]T Triplet. [c]D Doublet. [d]M Multiplet.

Figure-5: Structure of an unknown impurity RRT 2.05

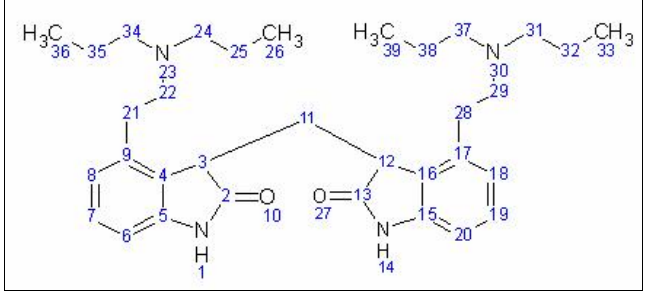


Figure-6: Mass fregmentation of an unknown degraded impurity RRT 2.05

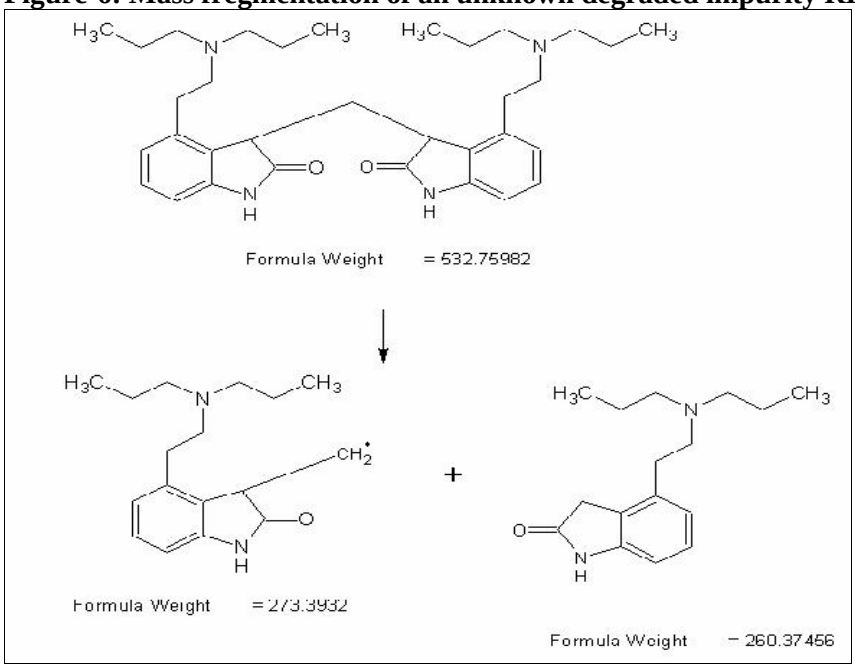


Figure-7(A): Mass spectra of impurity with RRT 2.05

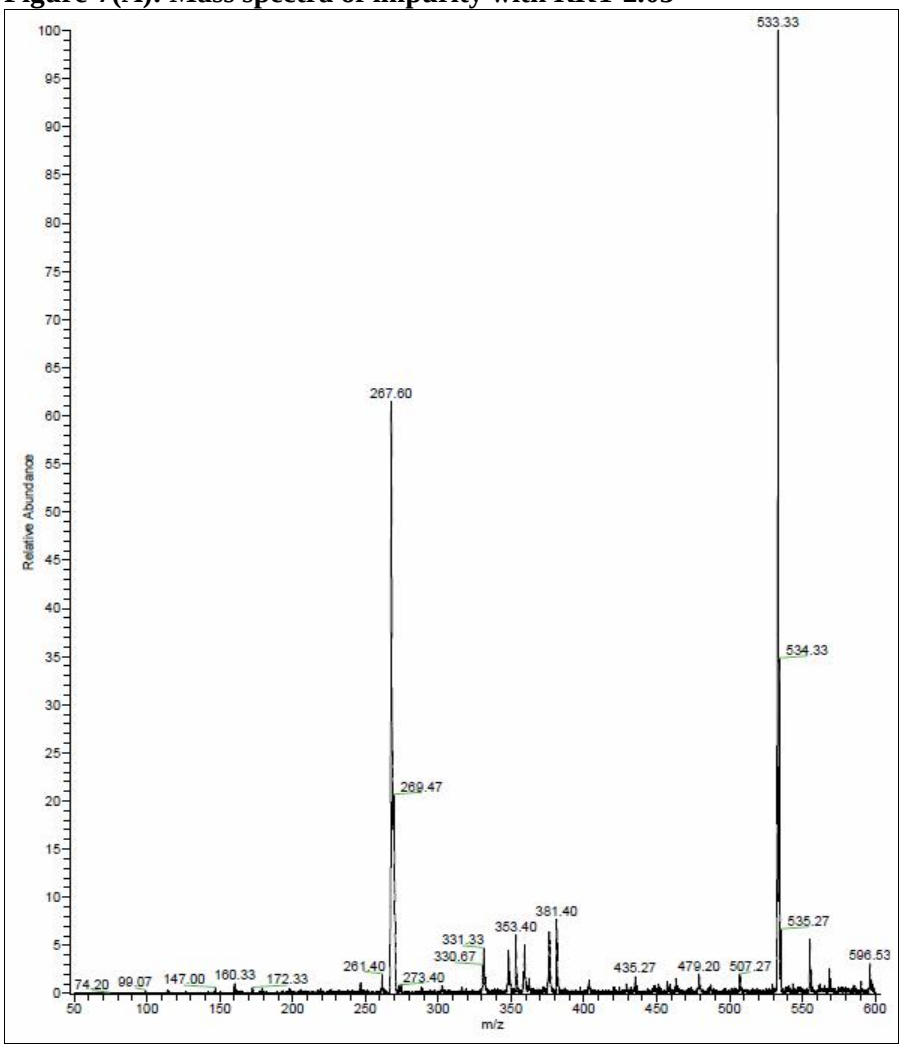
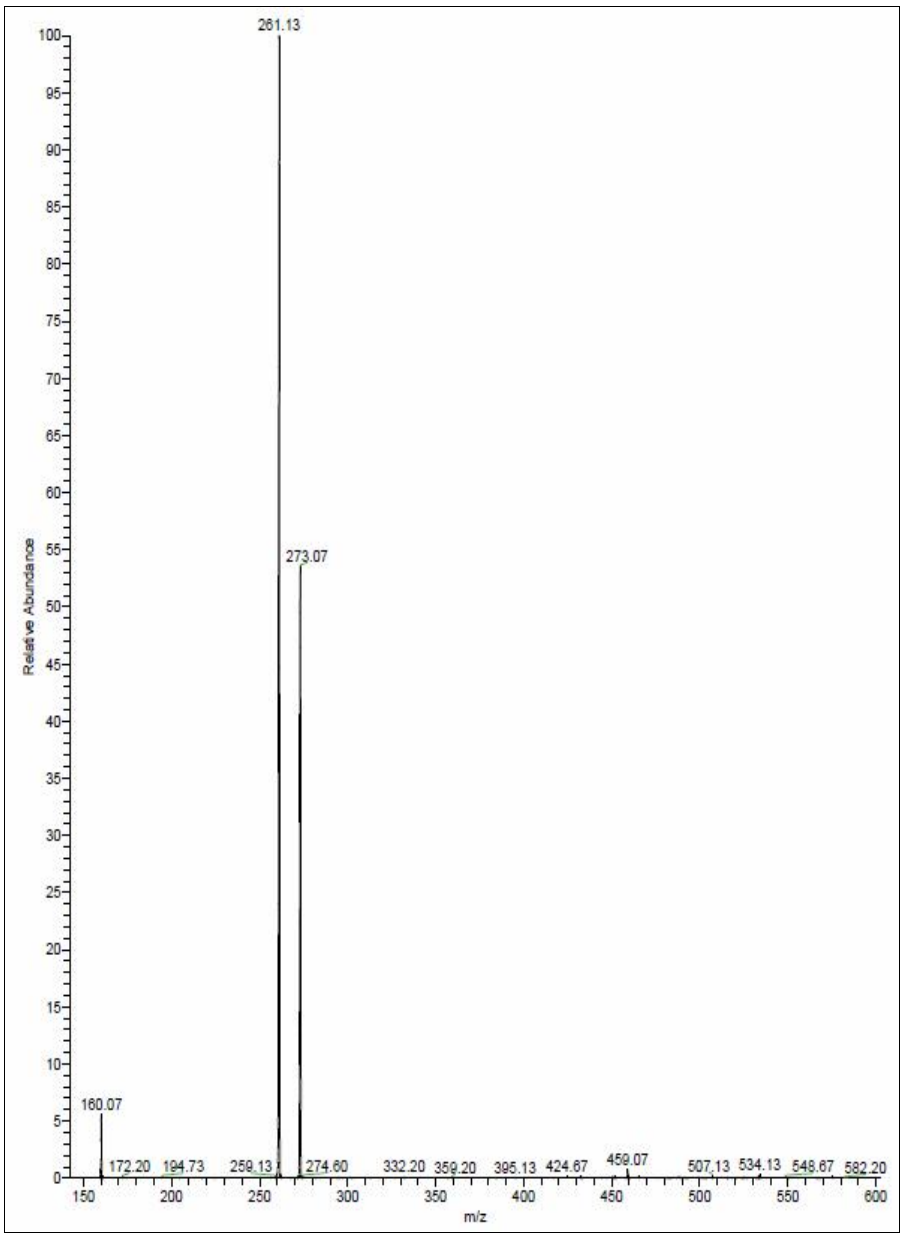


Figure-7 (B): MS-MS spectra of impurity with RRT 2.05



5.0 Acknowledgement

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6.0 References

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