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Identification and characterization of process related impurities in pramipexole dihydrochloride monohydrate

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ABSTRACT

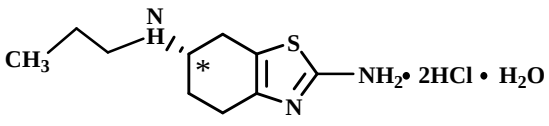
Pramipexole dihydrochloride monohydrate is used for treatment of idiopathic Parkinson's disease. During routine monitoring of the drug samples by HPLC, two impurities of pramipexole were observed. The molecular weights of the impurities were determined by LC-MS. The structures were postulated to be (6S)-(-)-2-amino-6-ethylamino-4,5,6,7-tetrahydrobenzo thiazole (ethyl pramipexole) and (6S)-(-)-2,6-di-(n-propylamino)-4,5,6,7-tetrahydrobenzothiazole (dipropyl pramipexole). These were synthesized subsequently, and characterized by NMR and IR. Their presence was confirmed by spiking into pramipexole sample and carrying out HPLC analysis. To our knowledge, ethyl pramipexole and dipropyl pramipexole have not been reported as process impurities elsewhere.

KEYWORDS

Pramipexole;
Impurities;
Liquid chromatography-Mass spectroscopy (LC-MS).

INTRODUCTION

Pramipexole dihydrochloride monohydrate, chemically known as (6S)-(-)-2-amino-6-(n-propylamino)-4,5,6,7-tetrahydrobenzothiazole dihydrochloride monohydrate (I), a tetrahydrobenzothiazole derivative, is a dopamine D3/D2 agonist. Pramipexole dihydrochloride (brand name: Mirapex®, Pharmacia and Upjhon Co., USA) is used for treatment of idiopathic Parkinson's disease. The precise mechanism of action for pramipexole as a drug for Parkinson's disease is unknown, although it is believed to be related to its ability to stimulate dopamine receptors in the striatum[1-4]. The synthesis of pramipexole was first disclosed in 1987 [5-6]. Pramipexole has also been described for treating schizophrenia and depression[7], as a neuroprotective agent[8], for the treatment of restless legs syndrome[9], in the treatment of addictive disorders[10] and in the treatment of CNS disorders[11-12].



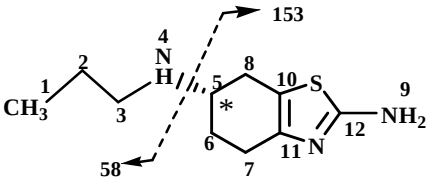
***asymmetric centre (I)**

Literature available was mainly regarding determination of pramipexole in human plasma with electrochemical detection, ultraviolet detection and with atmospheric pressure chemical ionization tandem mass spectrometry[13-14]. Chiral liquid chromatographic method for the enantiomeric separation was also avail-

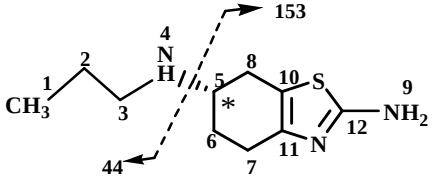
hydrogen phosphate solution and 2ml of triethyl amine, pH adjusted to 6.0 with orthophophoric acid) and 2 volumes of acetonitrile. The mobile phase (B) consisted of acetonitrile. Flow rate was kept at 1.0 ml/min and the column eluent was monitored at 260nm. Pump mode was gradient and was as follows, time (min)/A (v/v):B (v/v); $T_{0.01}$/100:0, T_{10}/97:3, T_{25}/80:20, T_{35}/65:35, T_{45}/60:40, T_{46}/100:0, T_{55}/100:0.

Liquid chromatography-mass spectroscopy

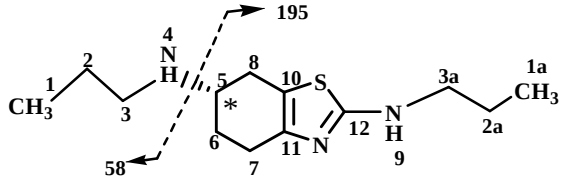
ESI mass spectra were recorded on Perkin Elmer



1(a) Chemical structure of pramipexole



1(b) Chemicla structure of Impurity-I (ethyl pramipexole)



1(c) Chemical structure of impurity-II (dipropyl pramipexole)

Note: Numbering is given for convenience of spectral interpretation and is not as per IUPAC nomenclature

Figure 1: Chemical structure of pramipexole and impurities

able[15]. To the best of our knowledge, ethyl pramipexole and dipropyl pramipexole impurities were not cited in literature to date.

During the analysis of different laboratory batches of pramipexole dihydrochloride monohydrate, two impurities were detected whose area percentage ranged from 0.05% to 0.1%. A thorough study has been undertaken to characterize these impurities, by spectroscopic techniques. In wake of regulatory requirements, all impurities above 0.05 %w/w need to be identified, and characterized[16]. Present study deals with identification, formation of impurities, their preparation, and characterization.

EXPERIMENTAL

Sample, chemicals and reagents

Pramipexole dihydrochloride monohydrate and impurities were synthesized in APL Research Centre (A Division of Aurobindo Pharma Ltd., Hyderabad, INDIA). All the reagents used for analysis were procured from Merck (India) limited. Distilled water was prepared by using Milli-Q water purification system (Millipore, Bedford, MA).

High performance liquid chromatography (Analytical conditions)

Chromatographic separations were performed on high performance liquid chromatograph, Waters alliance 2695 separations module equipped with 2996 photodiode array detector and Empower pro data handling system[Waters Corporation, MILFORD, MA01757, USA]. Separations were achieved on YMC Pack C8 column with dimensions of 250 mm×4.6mm i.d, 5 μm particle size maintained at 30°C. The mobile phase (A) consisted of 98 volumes of buffer (10mM diammonium

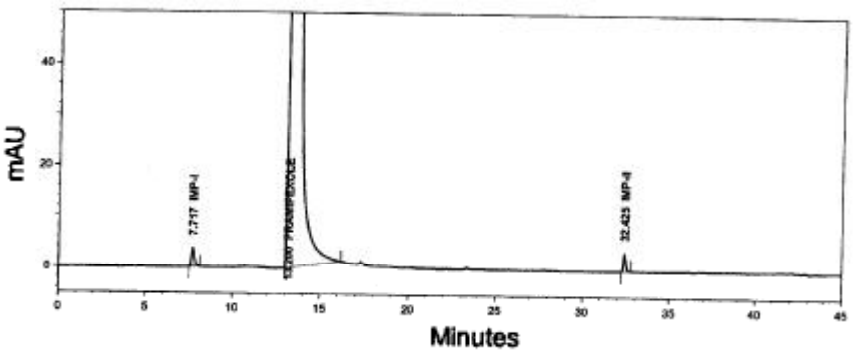


Figure 2: LC-Chromatogram of pramipexole dihydrochloride monohydrate sample spiked with impurities

triple quadrupole mass spectrometer (API 2000, PE SCIEX) coupled with Shimadzu HPLC equipped with SPD 10A VP UV-Vis detector and LC 10AT VP pumps. Analyst software was used for data acquisition and data processing. The turbo ion spray voltage was maintained at 5.5 Kv and temperature was set at 375°C. The auxiliary gas and sheath gas used was high pure nitrogen. Zero air was used as Nebuliser gas. LC-MS spectra were acquired from m/z 70-700 in 0.1 amu steps with 2.0 sec dwell time. As inorganic salt buffers can not be introduced into the LC-MS system, the analytical method is modified to volatile buffers with same elution profile and LC-MS analysis of the crude sample was carried out using Inertsil ODS 3V column with dimensions of 250mm × 4.6mm, 5.0 µm particle size. The mobile phase consisted of 10mM ammonium acetate solution, pH adjusted to 5.0 with glacial acetic acid (A) and acetonitrile: methanol:[1:1, v/v] (B). Flow rate was 1.0 ml/min. Pump mode was gradient and was as follows, time (min)/A (v/v):B (v/v); $T_{0.01}$/97:3, T_{15}/85:15, T_{25}/70:30, T_{35}/50:50, T_{36}/97:3, T_{45}/97:3.

NMR spectroscopy

The ^{1}H, ^{13}C(proton decoupled) experiments were performed on a Bruker 300MHz (Avance DPX-300) NMR spectrometer using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard.

IR spectroscopy

The IR spectra were recorded in the solid state as KBr pellet using Perkin Elmer instrument, model-spectrum one.

RESULTS AND DISCUSSION

Detection and identification

Sample solutions of pramipexole dihydrochloride monohydrate were prepared and injected into the analytical LC using the solvent system is described earlier. Two impurities eluted at relative retention times (RRT) 0.59 and 2.46 respectively with respect to pramipexole, whose retention time is 13.2 minutes. A typical chromatogram is shown in figure 2. The same samples were subjected to LC-MS using conditions as described in section 2.3 to identify the molecular mass (m/z) of the impurities. The m/z values of the impurities recorded in positive ion mode were 198 for impurity-I eluted at RRT-0.59, and 254 for impurity-II eluted at RRT-2.46.

ESI mass spectrum of pramipexole in positive ion mode showed a molecular ion peak at m/z 212[$(MH)^+$] indicating the molecular weight of the compound as 211. The major fragment ion peak was observed at m/z 153, reflecting 2-amino-4,5,6,7-tetrahydrobenzothiazole moiety with cleavage of n-propylamine chain as depicted in figure 1a. The m/z value difference between the pramipexole mass and its major fragment ion was 58 mass units, reflecting propylamine chain.

ESI mass spectrum of impurity-I in positive ion mode showed a molecular ion peak at m/z 198[$(MH)^+$] indicating the molecular weight of the compound as 197, showed 14 mass units less than pramipexole. The major fragment ion peak was observed at m/z 153, reflecting 2-amino-4,5,6,7-tetrahydrobenzothiazole moiety. The m/z value difference between the impurity mass and its major fragment ion was 44 mass units, reflecting ethylamine chain i.e., one methylene group (14 mass units) less than propylamine chain of pramipexole. From this data, ethyl pramipexole structure was proposed for impurity-I as shown in figure 1b.

ESI mass spectrum of impurity - II in positive ion mode showed a molecular ion peak at m/z 254[$(MH)^+$] indicating the molecular weight of the compound as 253, showed 42 mass units more than pramipexole. The major fragment ion peak of this impurity was observed at m/z 195(Figure 1c) compared to pramipexole major fragment ion peak observed at m/z 158(Figure 1a). This 42 mass units difference reflect propyl chain binding to amino function of 2-amino-4,5,6,7-tetrahydrobenzo thiazole moiety. From this data, dipropyl pramipexole structure was proposed for impurity-II as indicated in figure 1c.

Based on the proposed structures, these impurities were synthesized, spectrally characterized by NMR, mass, IR data, and also confirmed by HPLC analysis via spiking these impurities into pramipexole sample.

Preparation of impurities

The proposed impurities were prepared and confirmed spectrally via NMR, mass and IR data. The comparative ^{1}H and ^{13}C NMR spectral data of pramipaxole, impurity - I and impurity - II is given in TABLE 1 and FT-IR spectral data for the same is given in TABLE 2. The spectral data of pramipaxole, impurity - I and impurity - II were recorded in free base form for spectral

TABLE 1: Comparative ^{1}H and ^{13}C NMR assignments for pramipexole and its impurities

Spectral data of pramipexole and impurities as free base were considered for spectral comparison							
	Pramipexole		Impurity I		Impurity II		
Position[a]	^{1}H(ppm) multiplicity	^{13}C (ppm)	^{1}H (ppm) multiplicity	^{13}C (ppm)	Position[a]	^{1}H (ppm) multiplicity	^{13}C (ppm)
1	0.87 (t, 3H)	12.8	1.01 (t, 3H)	16.3	1, 1a	0.84 – 0.90 (2t, 6H)	12.3, 12.7
2	1.40 (sextet, 2H)	24.0	-	-	2, 2a	1.37 – 1.55 (2sextet, 4H)	22.9, 24.0
3	2.74 (distorted triplet, 2H)	49.6	2.57 (distorted triplet, 2H)	41.7	3,3a	2.50 & 3.09 (2 distorted triplet, 4H)	46.9, 49.6
4	1.49 (m, 1H)	-	1.48 (m, 1H)	-	4	1.48 (m, 1H)	-
5	2.78 (m, 1H)	54.7	2.80 (m, 1H)	54.6	5	2.75 (m, 1H)	54.7
6	1.51 &1.89 (2m, 2H)	30.1	1.47 & 1.89 (2m, 2H)	30.1	6	1.52 & 1.90 (2m, 2H)	30.1
7	2.40 & 2.53 (2m, 2H)	30.5	2.41 & 2.70 (2m, 2H)	30.4	8	2.44 & 2.71 (2m, 2H)	30.5
8	2.18 & 2.35 (2m, 2H)	25.7	2.21 & 2.35 (2m, 2H)	25.7	7	2.20 & 2.42 (2m, 2H)	25.9
9	6.59 (brs, 2H)	-	6.60 (s, 2H)	-	9	7.23 (t, 1H)	-
10	-	114.0	-	114.0	10	-	113.1
11	-	145.3	-	145.3	11	-	145.1
12	-	166.7	-	166.7	12	-	167.1

s, singlet; m, multiplet; t, triplet; q, quartet; brs, broad singlet, [a] Refer structural formula for numbering (Figure 1)

TABLE 2: FT-IR spectral data for Pramipexole and its impurities

Spectral data of pramipexole and impurities as free base were considered for spectral comparison			
S.no.	Compound	IR (KBr) absorption bands, v (Cm^{-1})	
1.	Pramipexole	3293, 3093(br)	NH stretch
		2957, 2922(m)	CH_3&CH_2 stretch
		1643(m) (primary)	NH bending
		1593(m)	C=N stretch
		1538(s)	NH bending (secondary)
		1466(m)	CH_2 bending
		1451, 1364(m)	CH_3 symmetric& asymmetric bending
		1121(s)	C-N stretch
2.	Impurity- I	3242(br)	NH stretch
		2965, 2918(m)	CH_3&CH_2 stretch
		1652(m) (primary)	NH bending
		1588(m)	C=N stretch
		1537(s)	NH bending (secondary)
		1468(m)	CH_2 bending
		1447, 1373(m)	CH_3 symmetric& asymmetric bending
		1118(s)	C-N stretch
3.	Impurity – II	3200(br)	NH stretch
		2956, 2931(m)	CH_3&CH_2 stretch
		1588(s)	C=N stretch
		1538(brs)	NH bending (secondary)
		1475(m)	CH_2 bending
		1455, 1365(m)	CH_3 symmetric& asymmetric bending
		1112(s)	C-N stretch

s, strong; m, medium; br, broad; brs, broad and strong.

comparisons.

Preparation of pramipexole

(6S)-(-)-2,6-Diamino-4,5,6,7-tetrahydrobenzo thiazole (III, Figure 3), was reacted with propionic anhydride and the isolated product (6S)-(-)-2-amino-6-propionamido-4,5,6,7-tetrahydrobenzothiazole (IV, Figure 3) was reduced with sodium borohydrate in the presence of BF_3 etherate to yield pramipexole base. The scheme for the synthesis of pramipaxole is shown if figure 3. $[\alpha]_D^{25}$: -93.8° , C = 1 in methanol.

Preparation of impurity- I (ethyl pramipexole)

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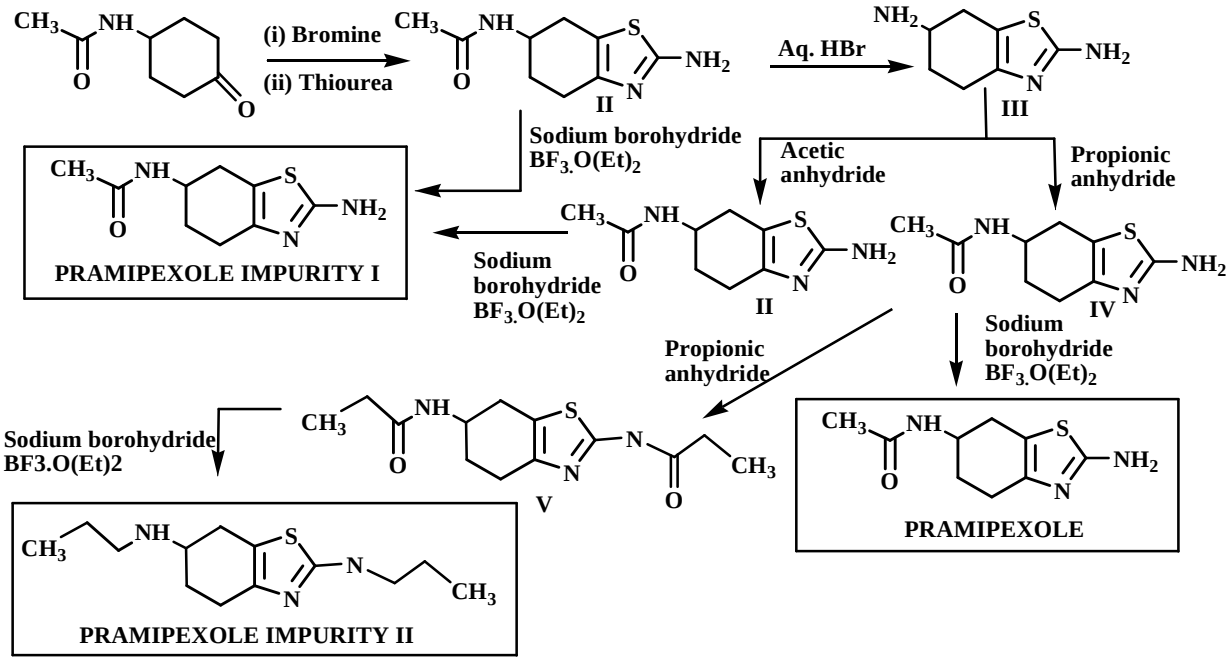

Figure 3: Scheme for the synthesis and formation of pramipexole and impurities

(6S)-(-)-2,6-Diamino-4,5,6,7-tetrahydrobenzo thiazole (III, Figure 3), was reacted with acetic anhydride and the isolated product 6-acetamido-2-amino-4,5,6,7-tetrahydrobenzothiazole (II, Figure 3) was reduced to yield impurity I. The chromatographic purity of the prepared impurity was 99.5% determined by injecting into the analytical LC using the solvent system is described earlier 3.3. In the ^{1}H NMR spectrum of pramipexole, the sextet at 1.40 ppm assigned to CH_2 at 2-position is absent in impurity-I. In N-CH_2-CH_3, the CH_2 adjacent to nitrogen was slightly shifted up field to 2.57 ppm, when compared to pramipexole. The triplet at 1.01 ppm clearly establishes the N-CH_2-CH_3 group in the molecule. In the ^{13}C NMR spectrum of pramipexole, the signal at 24.0 ppm assigned to CH_2 at 2-position was absent in impurity-I. The signal at 16.3 ppm is assignable to N-CH_2-CH_3 and the signal at 41.7 ppm is assignable to N-CH_2-CH_3 reflecting slight shift of δ -values to up field when compared to pramipexole. $[\alpha]_D^{25}$: -94.0° , C = 1 in methanol. The above spectral data confirms the impurity as (6S)-(-)-2-amino-6-ethylamino-4,5,6,7-tetrahydrobenzothiazole (ethyl pramipexole) with molecular formula $C_9H_{15}N_3S$ and molecular weight 197.

Preparation of impurity - II (dipropyl pramipexole)

(6S)-(-)-2,6-Diamino-4,5,6,7-tetrahydrobenzo thiazole(III, Figure 3), was reacted with propionic anhydride and the isolated product (6S)-(-)-2,6-dipropionamido-4,5,6,7-tetrahydrobenzothiazole(V, Figure 3) was reduced to yield impurity II. The chromatographic purity of the prepared impurity was 99.5% determined by injecting into the analytical LC using the solvent system is dscribed earlier. In the ^{1}H NMR spectrum of pramipexole, the singlet at 6.59 ppm assigned to -NH_2 at 9-position is shifted downfield to 7.23 ppm in impurity-II with a triplet, indicating -NH- at 9-position. Also the two triplets at 0.84-0.90 ppm in this impurity compared to single triplet at 0.87 ppm in pramipexole; two sexlets at 1.37-1.55 ppm in this impurity compared to single quartet at 1.40 ppm in pramipexole; and two distorted triplets at 2.50 and 3.09 ppm in this impurity compared to single multiplet at 2.74 ppm in pramipexole clearly indicates the presence of two n-propyl groups in the molecule.$[\alpha]_D^{25}$: -84.5° , C = 1 in methanol. The above spectral data confirms the impurity as (6S)-(-)-2,6-di-(n-propylamino)-4,5,6,7-tetrahydrobenzothiazole (dipropyl pramipexole) with molecular formula $C_{13}H_{23}N_3S$ and molecular weight 253.

Formation of impurities

1. Formation of impurity-I

Sodium borohydrate reduction of 6-acetamido-2-

amino-4,5,6,7-tetrahydrobenzothiazole in the presence of BF_3 etherate (II, Figure 3) present in (6S)-(-)-2,6-diamino-4,5,6,7-tetrahydrobenzothiazole (III, Figure 3), results in ethyl pramipexole. The other mechanism is the presence of acetic anhydride as impurity in propionic anhydride, during the reaction from (6S)-(-)-2,6-diamino-4,5,6,7-tetrahydrobenzothiazole (III, Figure 3) to (6S)-(-)-2-amino-6-propionamido-4,5,6,7-tetrahydrobenzothiazole (IV, Figure 3), gives the corresponding 6-acetamido-2-amino-4,5,6,7-tetrahydro benzothiazole(II, Figure 3), which after reduction results in ethyl pramipexole (Impurity - I) (1b, Figure 1).

2. Formation of impurity-II

The acylation of both amino groups in (6S)-(-)-2,6-diamino-4,5,6,7-tetrahydrobenzothiazole (III, Figure 3) results in (6S)-(-)-2,6-dipropionamido-4,5,6,7-tetrahydrobenzothiazole (V, Figure 3), which after Sodium borohydrate reduction in the presence of BF_3 etherate results in dipropyl pramipexole (Impurity -II) (1c, Figure 1).

CONCLUSION

Two process impurities found in Pramipexole dihydrochloride monohydrate bulk drug samples during regular monitoring by HPLC were identified by LCMS. These impurities were identified, prepared, and characterized by spectroscopic techniques viz., IR, NMR, and MS.

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