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Journal of Pharmaceutical and Biomedical Analysis 46 (2008) 165–169
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Identification, isolation and characterization of potential degradation product in risperidone tablets

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Received 20 March 2007; received in revised form 8 August 2007; accepted 8 August 2007
Available online 14 August 2007

Abstract

One unknown impurity (degradation product) present at a level below 0.1% in the initial samples increased to a level of 0.5% in 6 M/40 °C/75% RH stability samples of risperidone tablets was detected by gradient reverse-phase high-performance liquid chromatography (HPLC). This impurity was isolated using reverse-phase preparative liquid chromatography. Based on the spectral data the structure of this impurity is characterized as 3-[2-[4-[6-fluoro-1,3-benzoxazol-2-yl]piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*] pyrimidin-4-one. Structural elucidation of this impurity by spectral data (^{1}H NMR, ^{13}C NMR, DEPT, MS and IR), formation and mechanism has been discussed in detail.

Keywords: Risperidone; Degradation product; Isolation; Preparative HPLC; Characterization

1. Introduction

Risperidone, an anti-psychotic drug is a benzisoxal derivative. It is chemically known as 3-[2-[4-[6-fluoro-1,2-benzisoxazol-3-yl]piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one.

Several impurities and degradation products which are known in the literature [1] are given in Fig. 3. Many methods were reported in the literature, dealt with determination of risperidone by HPLC in human plasma [2–4], quantitative determination of risperidone and 9-hydroxy risperidone in human plasma by LC–MS [5–7], separation of psychotropic drugs and respective metabolites by LC [8], HPLC and thin layer densitometric methods for the determination of risperidone in bulk drug and in tablets [9], isolation and characterization of degradation products, risperidone *N*-oxide and 9-hydroxy risperidone [10], structural studies of impurities of risperidone by hyphenated techniques [11]. As per the regulatory requirements, the impurity profile study has to be carried out for any final product [12].

This paper describes the identification, isolation and characterization of unknown impurity (degradation product) formed in risperidone tablets. Though many impurities and degradation products were reported in the literature, identification, isolation and characterization of the light/heat sensitive degradation product is not reported to the best of our knowledge.

2. Experimental

2.1. Samples

The investigated samples, risperidone tablets were formulated in APL Research Centre (A unit of Aurobindo Pharma Limited, Hyderabad, India). All known impurities were synthesized in chemical research department of APL Research Centre. Reagents used for analysis i.e., ammonium acetate (GR grade), orthophosphoric acid (~85% (w/w), AR grade), acetonitrile (HPLC grade), methanol (HPLC grade) were procured from Merck (India) Limited. Milli-Q grade water was used for the analysis and isolation.

2.2. High-performance liquid chromatography

A Waters 2695 separation module equipped with 2996 photo diode array detector with Empower pro data handling system

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doi:10.1016/j.jpba.2007.08.008

[Waters Corporation, Milford, MA, USA] was used. The analysis was carried out on Hypersil BDS C18, 100 mm long, 4.6 mm i.d., 3 μm particle diameter column. Mobile phase A was prepared by dissolving 5.0 g of ammonium acetate in 1000 ml of water. Mobile phase B was a degassed mixture of methanol and acetonitrile in the ratio of 80:20 v/v. UV detection was carried out at 260 nm and flow rate was kept at 0.8 ml/min. Column oven temperature was maintained at 45 °C. Data acquisition time was 50 min. Pump mode was gradient and the program was as follows: time (min)/A (v/v):B (v/v); $T_{0.01}$/80:20, $T_{15.0}$/70:30, $T_{25.0}$/60:40, $T_{35.0}$/50:50, $T_{45.0}$/30:70, $T_{50.0}$/30:70, $T_{51.0}$/80:20.

2.3. Preparative liquid chromatography

A Shimadzu LC-8A preparative liquid chromatograph equipped with SPD-10A VP, UV–vis detector (Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan) was used. Hyperprep HS C18 (250 mm long × 21.2 mm i.d.) preparative column packed with 8 μm particle size was employed for isolation of impurity. The mobile phase consisted of (A) 0.2 M ammonium acetate solution and (B) methanol:acetonitrile (1:1). Flow rate was kept at 20 ml/min and UV detection was carried out at 260 nm. The gradient program was as follows: time (min)/A (v/v):B (v/v); $T_{0.01}$/100:0, $T_{20.0}$/90:10, $T_{35.0}$/80:20, $T_{50.0}$/50:50, $T_{60.0}$/40:60.

2.4. NMR spectroscopy

The ^{1}H NMR, ^{13}C NMR (proton decoupled) and DEPT spectra were recorded on Bruker 300 MHz nuclear magnetic resonance spectrometer using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard.

2.5. Mass spectrometry

Mass spectra were recorded on PE SCIEX-API 2000 mass spectrometer equipped with a turboionspray interface at 375 °C. Detection of ions was performed in electrospray ionisation, positive ion mode.

2.6. FT-IR spectroscopy

FT-IR spectra were recorded as KBr pellet on Perkin-Elmer instrument model—spectrum one.

2.7. Enrichment of degradation product

Risperidone tablets powder was kept in an autoclave at 121 °C/15 lb pressure/30 min for three cycles. Autoclaved sample is analyzed by the HPLC method mentioned in Section 2.2. The degradation product is enriched to about 4.0% by area normalization.

2.8. Isolation of impurity (degradation product) by preparative HPLC

Risperidone tablets powder which is equivalent to 1.0 g of risperidone drug was taken into a beaker, added about 150 ml of methanol and mixed thoroughly. Then added about 150 ml of water and sonicated for 1 h. The mixture was filtered under vacuum using buckner funnel. The filtrate was concentrated on rotavapour to remove methanol. Then the concentrated filtrate was loaded into the preparative column using the conditions mentioned in Section 2.3. Fractions collected were analyzed by analytical HPLC as per the conditions mentioned in Section 2.2. Fractions of >95% were pooled together, concentrated on rotavapour to remove solvent mixture. Concentrated fractions were passed through the preparative column by using water:acetonitrile (50:50) as mobile phase to remove the buffer used for isolation. Again the eluate was concentrated using rotavapour to remove acetonitrile. The aqueous solution was lyophilized using freeze dryer (Virtis advantage 2XL). The impurity was obtained as pale yellow powder and the chromatographic purity is 96.0%, determined by the HPLC method mentioned in Section 2.2.

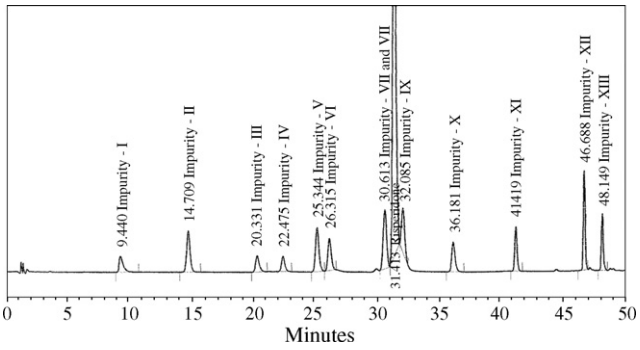

Fig. 1. LC-chromatogram of risperidone tablets spiked with known impurities.

3. Results and discussion

3.1. Detection of impurity

An analytical LC-chromatogram of a laboratory batch of risperidone tablets spiked with all known impurities is given in Fig. 1. The resolution mixture chromatogram of risperidone tablets spiked with unknown impurity (degradation product) was shown in Fig. 2. All the impurities were well resolved from risperidone peak. Relative retention time of degradation prod-

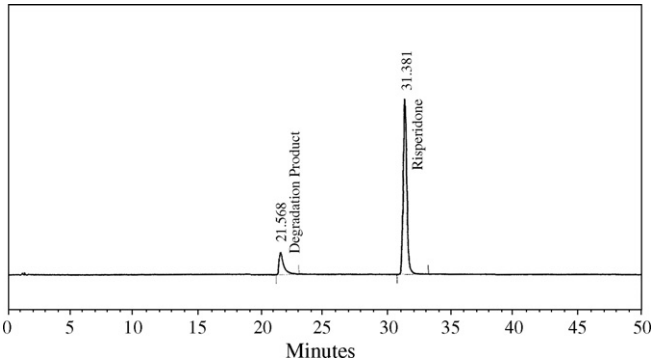

Fig. 2. LC-chromatogram of risperidone tablets spiked with degradation product.

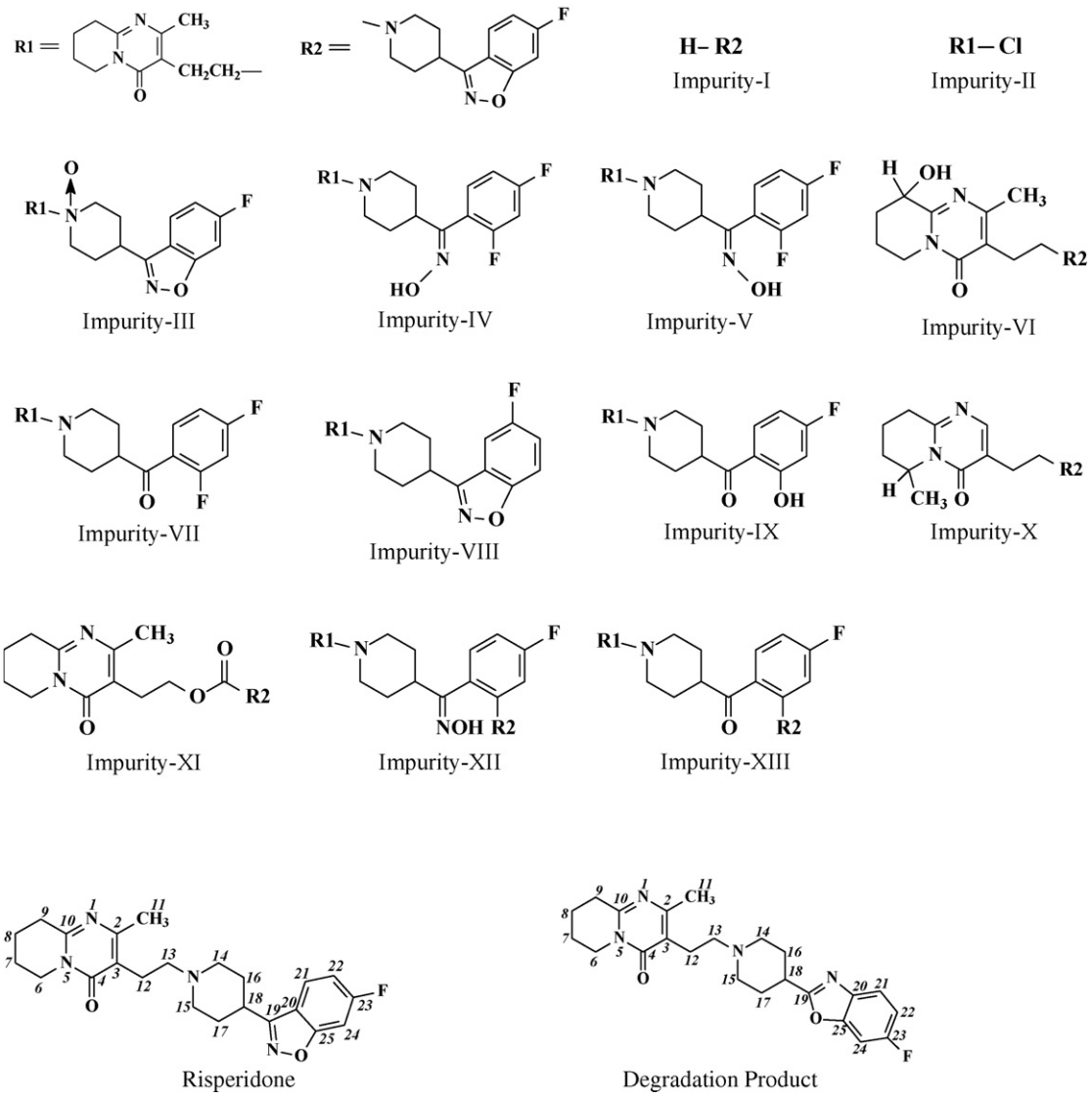

Fig. 3. Chemical structures of risperidone and impurities.

uct with respect to risperidone is about 0.68. Chemical structures of risperidone and degradation product are shown in Fig. 3.

3.2. Structural elucidation of impurity (degradation product)

The electrospray ionization (ESI) mass spectrum of this impurity (RRT∼0.68) exhibited a molecular ion peak at *m*/*z*, 411 amu [$(M\text{H})^+$] in positive ion mode, which is same as that of risperidone. Number of protons and carbon signals obtained in NMR spectra of impurity are same as that of signals present in risperidone. Major MS/MS fragmentation peak is at *m*/*z* 191 amu and is similar to risperidone. This information suggested that the degradation product is likely to be an isomer of risperidone. In comparison to risperidone NMR signals, there is no shift observed in the signals corresponding to tetrahydropyridopyrimidinone ring, but appreciable upfield shift is observed in the signals corresponding to benzisoxazol ring and down-field shift is observed in CH_2 and CH protons of piperidinyl ring. Methylene protons of piperidinyl ring have shifted from 30.9 ppm to 20.8 ppm, N–CH_2 signals of piperidinyl ring have shifted from 53.7 ppm to 56.6 ppm and CH signal of piperidinyl ring has shifted from 34.4 ppm to 27.2 ppm in ^{13}C NMR spectrum. Major down-field shift is observed in quaternary carbon of isoxazole ring (position 19 in Fig. 3) from 156.0 ppm to 185.4 ppm. In IR absorption spectrum of degradation product, the absorption bands corresponding to major functional groups are similar to risperidone. IR (KBr) absorption bands for impurity (cm^{-1}) are 2960 (aliphatic CH stretch), 1651 (N–CO stretch), 1600, 1538 (C=C and C=N stretch), 1413 (CH_2 bend), 1274, 1194 (C–N stretch), 980 (aryl CH out-of-plane bend). From the spectral data, the structure of this impurity is confirmed as 3-[2-[4-[6-fluoro-1,3-benzoxazol-2-yl]piperidin-1-yl]-ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one with molecular formula $C_{23}H_{27}FN_4O_2$ and molecular weight 410.5.

Table 1
NMR assignments of risperidone and its degradation product

Position[a]	Risperidone ^{1}H, δ (ppm) multiplicity	^{13}C, δ (ppm)	DEPT	Degradation product ^{1}H, δ (ppm) multiplicity	^{13}C, δ (ppm)	DEPT
2	–	158.6	–	–	160.0	–
3	–	117.5	–	–	116.0	–
4	–	164.1	–	–	174.0	–
6	3.78 (t, 2H)	43.0	CH_2	3.80 (t, 2H)	43.0	CH_2
7	1.75–1.85 (m, 4H)	22.3	CH_2	1.77–1.89 (m, 4H)	22.0	CH_2
8		19.5	CH_2		19.2	CH_2
9	2.76 (t, 2H)	31.7	CH_2	2.79 (t, 2H)	31.6	CH_2
10	–	161.4	–	–	162.4	–
11	2.21 (s, 3H)	21.8	CH_3	2.29 (s, 3H)	21.8	CH_3
12	2.38–2.41 (m, 2H)	24.1	CH_2	3.06–3.09 (m, 2H)	22.8	CH_2
13	2.58–2.61 (m, 2H)	57.0	CH_2	3.63–3.66 (m, 2H)	62.8	CH_2
14	2.17 and 3.02 (2m, 4H)	53.7	$2 \times CH_2$	3.40 and 3.90 (2m, 4H)	56.6	$2 \times CH_2$
15						
16	2.08 and 2.30 (2brm, 4H)	30.9	$2 \times CH_2$	1.89 and 2.10 (2brm, 4H)	20.8	$2 \times CH_2$
17						
18	3.08–3.20 (m, 1H)	34.4	CH	4.41 (m, 1H)	27.2	CH
19	–	156.0	–	–	185.4	–
20	–	119.5	–	–	116.2	
21	7.99–8.04 (dd (J = 8.8 Hz), 1H)	124.7 (d, 11.0 Hz)	CH	7.33–7.39 (dd (J = 8.0 Hz), 1H)	131.0 (d, 13.4 Hz)	CH
22	7.25–7.31 (ddd (J = 8.8 Hz, 1.92 Hz), 1H)	113.2 (d, 25.0 Hz)	CH	6.09–6.14 (ddd, (J = 8.0 Hz), 1H)	106.2 (d, 17.0 Hz)	CH
23	–	162.6, 165.9 (J = 245 Hz)	–	–	165.6, 169.0 (J = 245 Hz)	–
24	7.67–7.71 (dd (J = 8.8 Hz, 1.92 Hz), 1H)	98.1 (d, 25 Hz)	CH	6.28–6.33 (dd, (J = 8.0 Hz), 1H)	100.6 (d, 23.0 Hz)	CH
25	–	165.9	–	–	158.3	–

s, singlet; d, doublet; t, triplet, dd, doublet of a doublet; m, multiplet; ddd, doublet of a double doublet, brm, broad multiplet; J, coupling constant.
[a] Refer structures for numbering (Fig. 3).

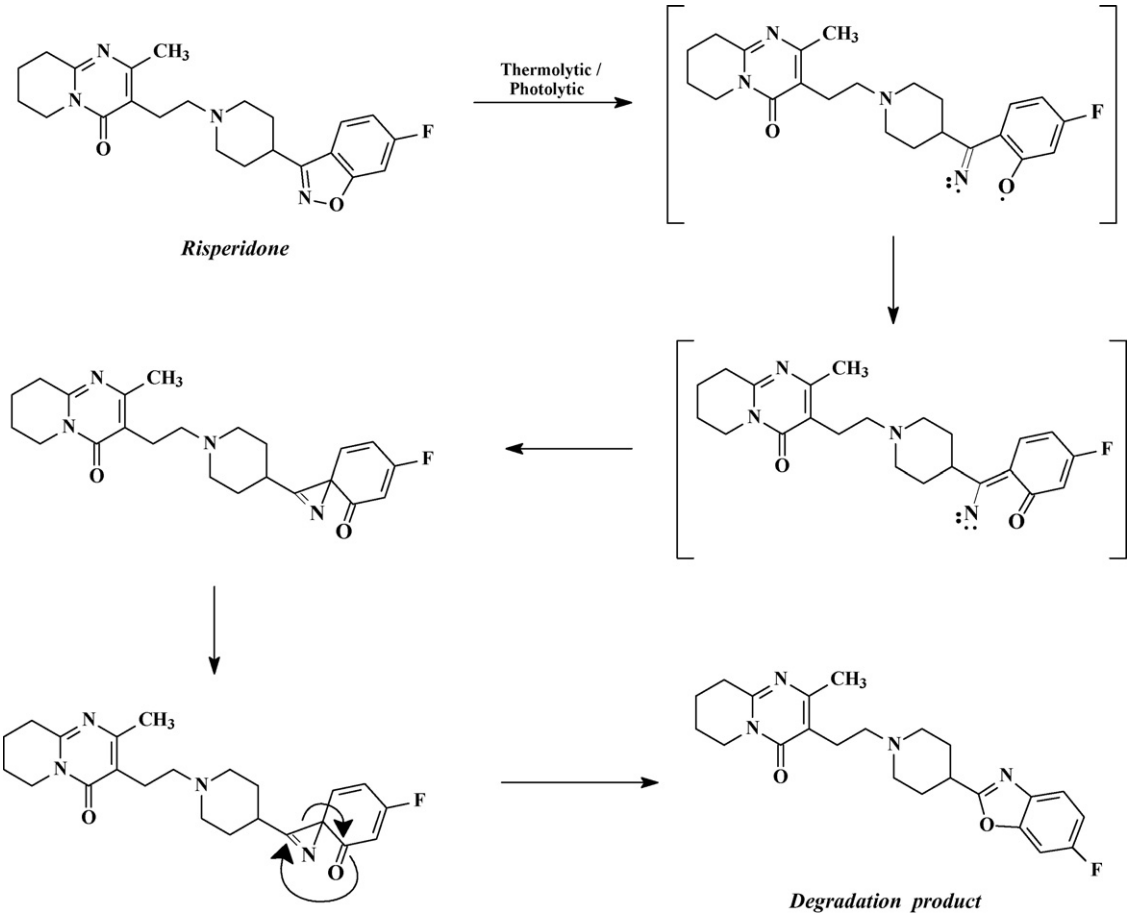

Fig. 4. Proposed degradation pathway.

The ^{1}H and ^{13}C NMR chemical shift values of risperidone and its degradation product are given in Table 1.

3.3. Formation and mechanism of degradation product

This impurity arises from heat- or light-induced rearrangement of isoxazole ring, present in risperidone, to the corresponding oxazole moiety. The proposed degradation pathway is shown in Fig. 4.

Acknowledgements

The authors gratefully acknowledge the management of Aurobindo Pharma Limited, for allowing us to carry out the present work. The authors are also thankful to the colleagues of Analytical Research Department (ARD), Formulation Analytical Research Department (FARD), Chemical Research Department (CRD) and Formulation Research Department (FRD) for their co-operation.

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