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Short communication

Identification and characterization of major degradation products of risperidone in bulk drug and pharmaceutical dosage forms

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Abstract

Acid, base and oxygen stability of risperidone, a novel anti-psychotic drug, has been evaluated storing the sample in solution phase. One of the major degradation products has been identified and characterized by using techniques namely IR, MS and NMR after isolation by preparative LC. The other major degradation product has been identified with help of MS/MS data and by co-eluting in analytical LC with the available standard. The effect of acid and base resulted in the formation of hydroxy risperidone and the effect of oxygen lead to the formation of N-oxide of risperidone. The two major degradation products in the dosage forms were also characterized as 9-hydroxy risperidone and N-oxide of risperidone, after enrichment through preparative LC, by LC–MS/MS and HPLC. Structural elucidation of degradation product leading to the formation of N-oxide of risperidone is discussed in detail.

Keywords: Risperidone; Degradation products; Isolation; Characterization; LC–MS/MS; NMR; IR

1. Introduction

Risperidone, a benzisoxal derivative, chemically known as 3-[2-[4-(6-fluoro-1,2-benzisoxalol-3-yl)piperidin-1-yl] ethyl]-2-methyl-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-4-one, a novel anti-psychotic drug. It acts by blocking both the serotinin $5HT2_A$ and dopamine D_2 receptors [1,2]. Many LC methods have been reported in the literature for the determination of risperidone and its active metabolite 9-hydroxy risperidone in plasma samples using UV and MS detections [3–7]. Few methods reported in the literature dealt with the analysis of pharmaceutical formulation of risperidone using first order derivative spectroscopy and reversed-phase liquid chromatography [8,9]. Determination of risperidone and 9-hydroxy risperidone by chromatography using electro-chemical detection [10] was reported for biological samples. None of the publications dealt with the separation, isolation and characterization of degradation products. Fragmentation pattern using electro spray ionization ion-trap for the drugs of saturated rings containing nitrogen atom is described in the literature [11]. In the present paper we have tried to identify and characterize the major degradation products of risperidone when it was subjected to acid, base and peroxide for 8 h.

2. Experimental

Risperidone was synthesized in MCD department of Torrent Research Center, India. 9-Hydroxy risperidone was purchased from Chempharm, USA. Stability samples of risperidone tablets (0.5 and 1.0 mg strength) kept at 60 °C for 1 month were received form formulation department of Torrent Research Center, India. Ammonium acetate, GR grade was obtained from E. Merck, India. Methanol, Acetonitrile of HPLC grade were procured from E. Merck, India. Purified water was collected through Milli-Q water purification system (Millipore, USA).

Chromatographic separations were performed on Thermofinnigan HPLC system consisting of Surveyor quaternary solvent delivery module, auto sampler and PDA detector.

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Table 1
Analytical LC conditions

Time (min)	% of solvent A	% of solvent AB
0	70	30
15	30	70
20	30	70
21	70	30
25	70	30

Solvent A: 70 mM ammonium acetate; solvent B: methanol.

Data was processed through Chromquest chromatography workstation software Version 1.63.

An Hypersil-BDS C18 column with dimensions of 100 mm × 4.6 mm i.d packed with 3.0 μm particle size was employed for separation. The gradient programme used was as per European Pharmacoepia with the mobile phase consisting of 70 mM ammonium acetate (A) and methanol (B) (for gradient conditions see Table 1). Flow rate was kept at 1.5 ml/min and the column eluent was monitored at 260 nm [12].

Preparative HPLC system used was a Shimadzu equipped with LC-8A solvent delivery module, CBM-10A communication bus module, SPD-10A UV/Vis detector and FRC-10A fraction collector. Sample was injected through FCV-130AL solenoid valve. Data was processed through LC-10 workstation software.

An Hypersil BDS-C18 HPLC column with dimensions 250 mm × 21.2 mm packed with 5.0 μm particle size was used for preparative work. The gradient conditions employed for the separations are shown in Table 2. Flow rate was kept at 25 ml/min and the column eluent was monitored at 260 nm.

The ^{1}H, ^{13}C, DEPT and COSY experiments were performed on a Bruker Avance DPX-400 MHz NMR spectrometer at 25 °C in DMSO-d_6. ^{1}H chemical shift values were reported on the δ scale in ppm relative to TMS ($\delta = 0.00$ ppm) and the ^{13}C chemical shift values were reported relative to DMSO-d_6 ($\delta = 39.5$ ppm) as internal standards respectively.

The IR spectra were recorded in the solid state as KBr as dispersion using Shimadzu FT-IR 8700 with DRS technique.

LC–MS/MS system used for recording MS spectra was a Thermofinnigan LCQ connected to Surveyor HPLC system. MS capillary voltage and temperature were set at 10 V and 225 °C, respectively. Spray voltage was maintained at 4.5 kV. The auxillary and sheath gas used was high pure nitrogen.

Table 2
LC–MS/MS conditions

Time (min)	% of solvent A	% of solvent AB
0	75	25
15	30	70
20	30	70
21	75	25
25	75	25

Solvent A: 30 mM ammonium acetate; solvent B: methanol.

2.1. Preparation of degradation samples of risperidone

2.1.1. Acid degradation

One gram of risperidone was taken in 25 ml of 0.5N HCl and refluxed for 3 h and cooled. After 3 h reflux and cooling the volume was made to 50 ml with 0.5N HCl.

2.1.2. Base degradation

One gram of risperidone was taken in 20 ml of Methanol and 20 ml of 0.1N NaOH and refluxed for 3 h and cooled. After 3 h reflux and cooling the volume was made to 50 ml with methanol.

2.1.3. Peroxide degradation

One gram of risperidone was taken in 20 ml of 3% (v/v) hydrogen peroxide in methanol and made up to volume with the same solvent and kept for 1 h.

2.2. Analysis of degradation samples by analytical LC

The degradation samples mentioned under Sections 2.1.1–2.1.3 were diluted to the required concentration and injected into analytical LC using the solvent system as described in Table 1. Major degradation products eluted at retention times approximately 8.5 and 11 while risperidone eluted at 12 min. A typical chromatogram is shown in Fig. 1.

2.3. LC–MS/MS analysis

LC–MS/MS analysis of the degradation samples was carried out using Hypersil-BDS C18 column with dimensions of 100 mm × 4.6 mm packed with 3.0 μm particle size. The mobile phase consisted of 30 mM ammonium acetate (A) and methanol (B). The gradient programme is described in Table 2. Since the buffer having a concentration of 30 mM was used in the analysis, column eluent was drained off wherever there was no peak of interest to avoid choking of the MS capillary. The molecular ions of the peaks due to the two major degradation were acquired in MS as well as MS/MS mode.

2.4. Isolation of degradation product(s) by preparative LC

The solvent system as described in Table 3 was used for the isolation of major degradation product of risperidone treated with hydrogen peroxide. Approximately 5.0 g of the degraded compound was loaded on to preparative LC and as many as 25 fractions were collected separately. Purities of all these fractions were checked on analytical LC and found to be in the range of 95–98%. The fractions having purity of more than 96% were pooled together. Solvent was evaporated under high vacuum using rotavapor Buchi Model B485. The remaining aqueous layer containing ammonium acetate salt was subjected to liquid–liquid extraction using chloroform. The organic layer was again concentrated under high vacuum to obtain the degradation product in solid form.

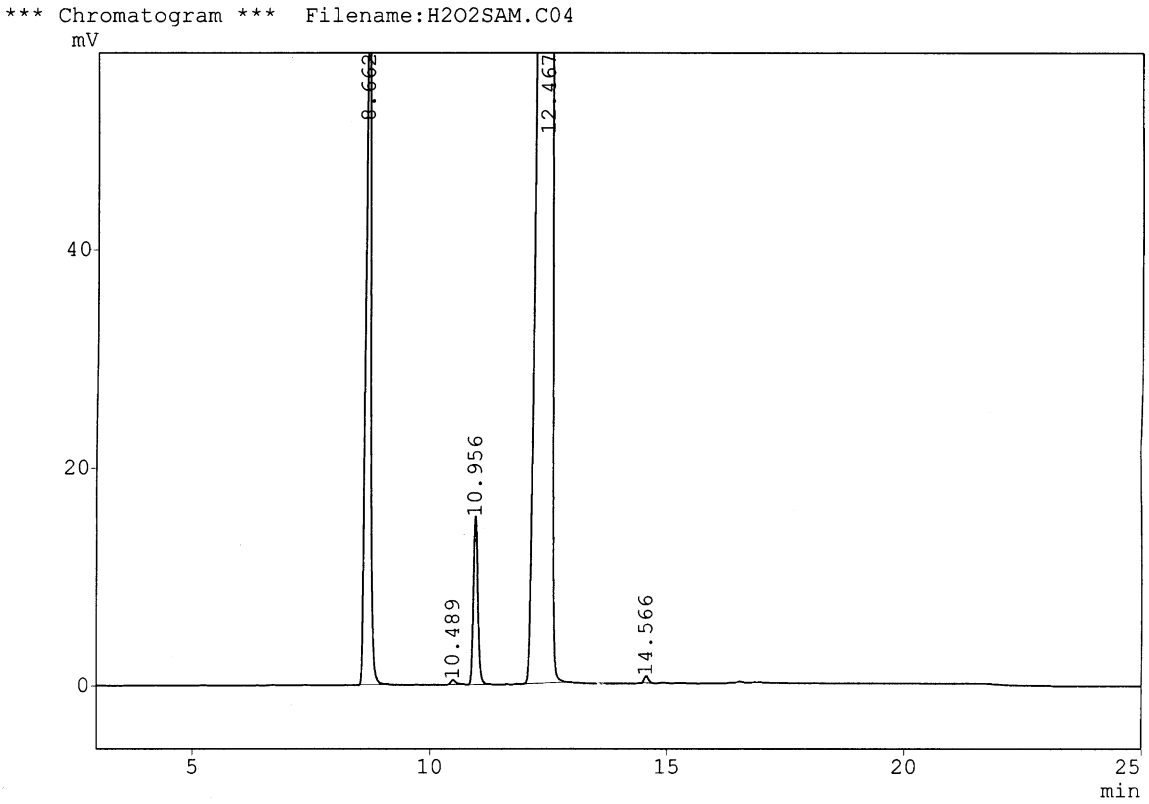

Fig. 1. Typical LC chromatogram of degraded sample risperidone bulk drug.

The solid thus obtained was reanalyzed on analytical LC and the purity of the same was found to be 97% which was relatively good enough for carrying out spectroscopic experiments.

2.5. Enrichment of degradation products in dosage forms

The analytical LC profile was checked for the tablet sample before taking up the enrichment work. The tablet sample was analyzed on a Symmetry C18 (250 mm × 4.6 mm), 5 μm HPLC column (Waters Corporation, USA) using a mobile phase consisting of 10 mM ammonium acetate and acetonitrile in a ratio of 75:25 (v/v). The flow rate was kept at 1.0 ml/min and the detection was carried out at 260 nm. A typical chromatogram of the tablet sample using these conditions is shown in Fig. 2a. Approximately 100 tablets of 1.0 mg strength kept at 60 °C for one month were ground well using mortar and pestle. The powder was dissolved in 50 ml of methanol. The resulting solution was centrifuged at 3000 rpm for 15 min. The supernatant layer was loaded on to preparative LC for enrichment of the required degradation products. The degradation products which were 0.2–0.5% initially were enriched up to 1.5% levels. The enriched fractions were then subjected to LC–MS/MS analysis.

Table 3
Preparative LC conditions

Time (min)	% of solvent A	% of solvent AB
0	70	30
20	65	35
25	60	40
30	50	50
50	40	60
60	70	30
70	70	30

Solvent A: 100 mM ammonium acetate; solvent B: methanol.

3. Results and discussion

3.1. Structural elucidation of degradation products

The molecular ions of both the major degradation products exhibited same molecular ion ($M + 1$) at m/z 427 amu which were 16 amu more than that of risperidone. Based on this information it was assumed that there was a presence of hydroxy group in the degradation products. To confirm the identity of these degraded products, 9-hydroxy risperidone standard was co-eluted with the degradation sample. Interestingly it was observed that the retention time of one of the peak was matching with the retention time of 9-hydroxy risperidone. The fragmentation pattern obtained in MS/MS data also supported that one of the degradation products was 9-hydroxy risperidone whose daughter ion was observed at

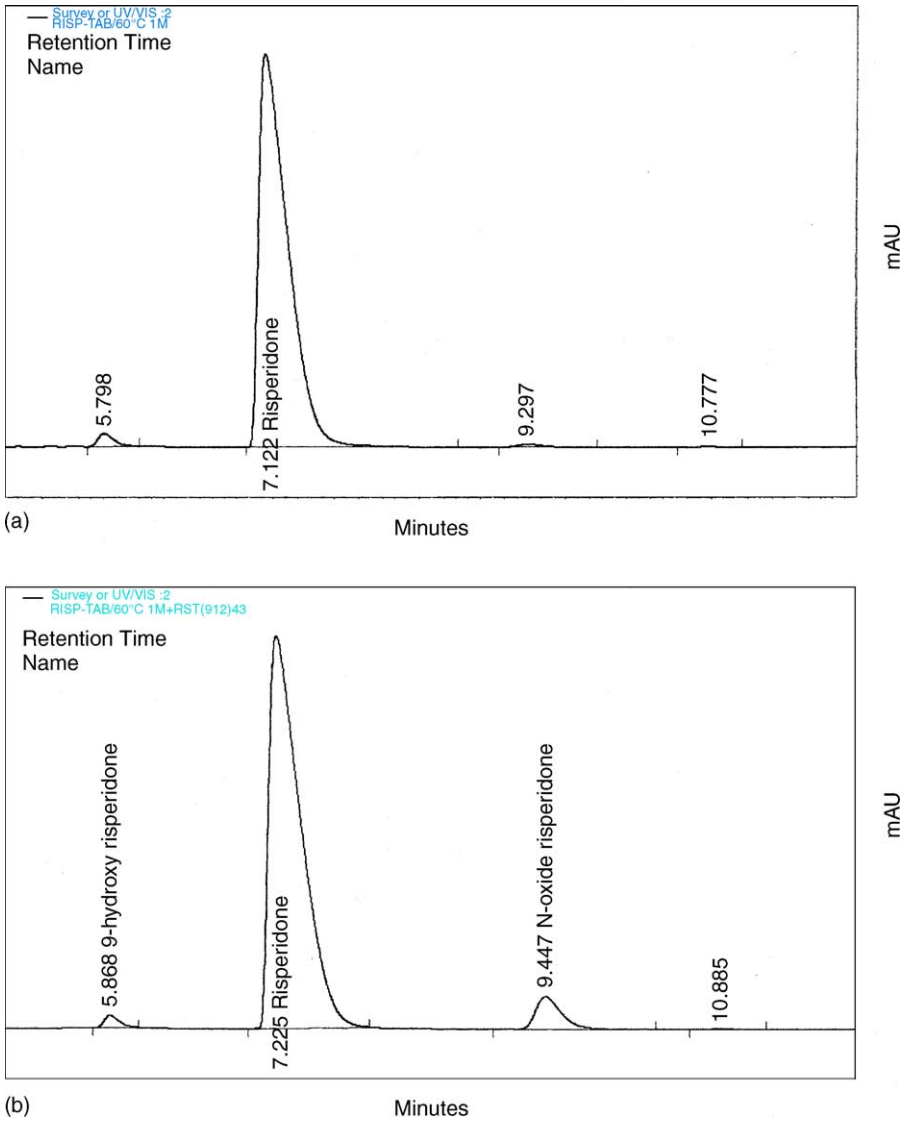

Fig. 2. (a) LC chromatogram of tablet sample of risperidone. (b) LC chromatogram of tablet sample of risperidone spiked with N-oxide risperidone.

m/*z* 207 amu in MS/MS experiment. On the other hand, the daughter ion of the second degradation product was 191 amu which was matching with the daughter ion of risperidone. This observation indicated that the incorporation of oxygen has not taken place on ring "a" (Fig. 3) which lead to the assumption that the incorporation of oxygen might be on one of the positions in the pyperidine ring "c". Since a stable daughter was observed for risperidone and the degradation product at *m*/*z* 191 amu, the possibility of insertion of oxygen on ring "a" was ruled out. Moreover, risperidone which has nitrogen containing saturated ring is resistant to fragmentation as reported in the literature [11]. This is the reason for a similar fragmentation pattern for both risperidone and degradation product. However, to confirm the structure of the degradation product, the degraded sample was subjected to isolation by preparative LC for further characterization.

The molecular ion ($M + 1$) of the isolated degradation product was 427 amu with a daughter ion at 191 amu. IR spectrum of the isolated degradation product did not exhibit any characteristic band due to hydroxy group stretching which indicated the absence of hydroxy group. ^{1}H NMR spectrum exhibited an equal number of protons when compared with risperidone. No proton signal was exchanged in D_2O exchange experiment which also supported the absence of hydroxy group in the degradation product. However, variations were observed in the ppm values (shifted slightly towards downfield) for the hydrogens present on pyperidine ring and the aliphatic side chain attached to nitrogen atom. The ^{13}C signals for the carbons of pyperidine ring and the aliphatic side chain attached to nitrogen were also shifted slightly towards downfield as well. The DEPT spectrum exhibited positive signals due to the presence of methyl and methane groups and negative signals due to the presence of methylene groups. Based on the above observations, the molecular formula of the isolated degradation product could be $C_{23}H_{27}FN_4O_3$. The above data can be rationalized in

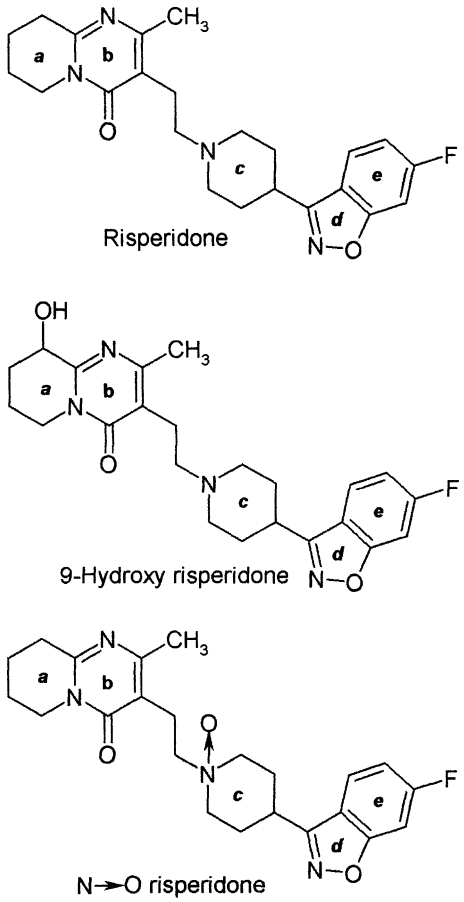

Fig. 3. Chemical structures of risperidone, 9-hydroxy risperidone and N-oxide risperidone.

terms of formation of N-oxide of risperidone. The chemical structures of risperidone, 9-hydroxy risperidone and N-oxide risperidone are shown in Fig. 3.

The synthesis of N-oxide impurity was carried out as per the procedure cited in the literature [13]. NMR, IR and MS data of this synthesized impurity was generated and found to match well with that of the isolated degradation product.

The synthesized N-oxide impurity was co-injected with the sample kept for peroxide degradation and found that the retention time was matching with the impurity of interest.

Since the LC analysis was carried out on a PDA detector, spectral matching was also performed for the isolated and synthesized impurities. The UV spectra of both the isolated and synthesized compounds matched well.

The two major degradation products in the enriched fractions of dosage forms exhibited same molecular ion at *m*/*z* 427 amu ($M + 1$) in LC–MS/MS analysis which were 16 amu more than that of risperidone. To confirm these degradation products, 9-hydroxy risperidone was co-eluted with the enriched fraction. Interestingly the retention time of one of the degradation products was matching with the retention time of 9-hydroxy risperidone. In the LC–MS/MS data where the daughter ion at *m*/*z* 207 amu also supported that one of the degradation products was due to the formation of 9-hydroxy risperidone in the stability samples of dosage forms. To identify the other degradation product, the synthesized N-oxide of risperidone was co-eluted with the enriched fraction where the retention time the other degradation product was matching with the retention time of N-oxide of risperidone (Fig. 2b). This observation was confirmed by MS/MS data where the daughter ions for both the compounds were observed at *m*/*z* 191 amu.

4. Conclusions

The major degradation products in risperidone bulk drug as well as pharmaceutical dosage forms were characterized by using spectroscopic techniques namely IR, NMR, MS and MS/MS. Out of the two degradation products, one degradation product was isolated by preparative LC for characterization studies.

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