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Structural studies of impurities of risperidone by hyphenated techniques☆

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Abstract

During the impurity profile of risperidone (RSP), a more polar impurity (RSP-1) and a more non-polar impurity (RSP-2) were detected in LC-MS with respect to risperidone. These impurities were isolated, enriched and were subjected to mass and NMR spectral studies. Based on the spectral data, RSP-1 and RSP-2 were characterized as risperidone N-oxide and 9-methylene risperidone, respectively. The formation of these impurities is rationalized. The structures of both the impurities were unambiguously confirmed by single crystal XRD Studies.

Keywords: Risperidone; Impurity; LC/MS/MS; Identification; Spectroscopy; Characterization; Single crystal; XRD studies

1. Introduction

Risperidone [1] is used to treat the symptoms of schizophrenia—a mental illness that causes disturbed or unusual thinking, loss of interest in life, and strong or inappropriate emotions. It works by interfering with the communication among nerves in the brain. The nerves communicate with one another by producing and releasing chemicals called neurotransmitters. The neurotransmitters attach to receptors on other nearby nerves and the attachment of the neurotransmitter causes changes in the cells that have the receptor on them. Resperidone blocks several of the receptors on nerves including dopamine type 2, serotonin type 2, and alpha 2 adrenergic receptors and this blocks communication among nerves. It is a new antipsychotic medication that probably has fewer side effects than many of the older medications.

The main metabolite of risperidone, 9-hydroxy risperidone [2] has similar activity as the parent compound. However, this was already identified and reported in the literature. One polar and one non-polar impurity were detected in the HPLC analysis of risperidone (Fig. 1). An LC-MS-MS method has been developed to identify these impurities. The characterization of these impurities was discussed. Recently, the N-oxide impurity has been identified in the formulation samples of risperidone [3]. The present work describes the total NMR spectral assignment of these impurities. The 9-methylene risperidone is reported for the first time.

2. Experimental

2.1. Samples and chemicals

The pharma grade sample of risperidone and synthesized impurities have been provided by Bulk Actives-IV, Dr. Reddy's Laboratories Ltd., Hyderabad, India. HPLC grade methanol was obtained from (Merck Co.) and ammonium acetate salt (Qualigens) were used in the analysis. Water used for preparing mobile phase was purified using Milli-Q plus purification system.

2.2. HPLC

A Shimadzu HPLC equipped with LC-10 AD pump and SPD-M10A VP diode array detector have been used. A Hypersil BDS (150 mm × 4.6 mm, 5 μm, Waters, USA) column was used for the separations. The column eluent was monitored at 260 nm. The mobile phases used are 0.065 M ammonium acetate and methanol. The mobile phase was filtered through a nylon membrane (pore size 0.45 μm). Chromatography was performed at room temperature at a flow rate of 1.5 mL min^{-1}. The HPLC conditions mentioned above have been used in the LC-MS analysis.

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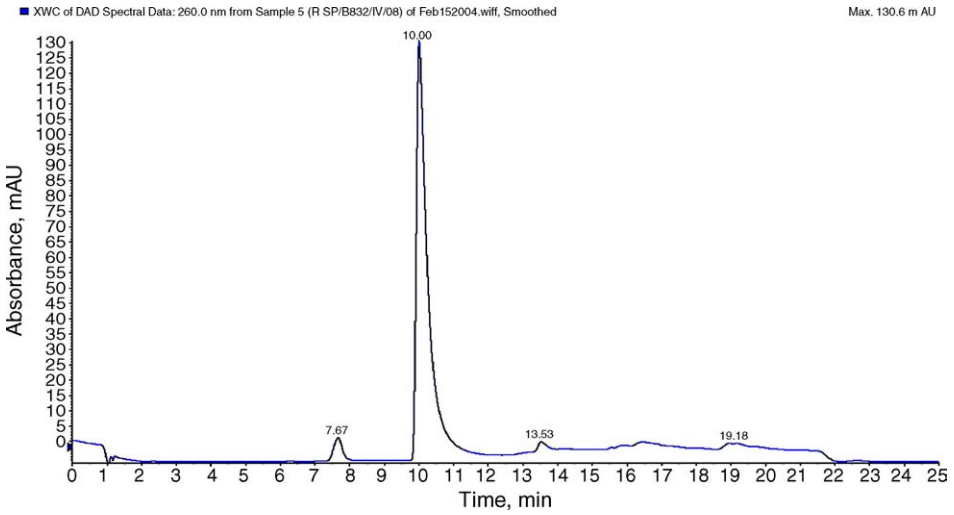

Fig. 1. HPLC analysis of risperidone (mobile phase: 0.065 M ammonium acetate and methanol).

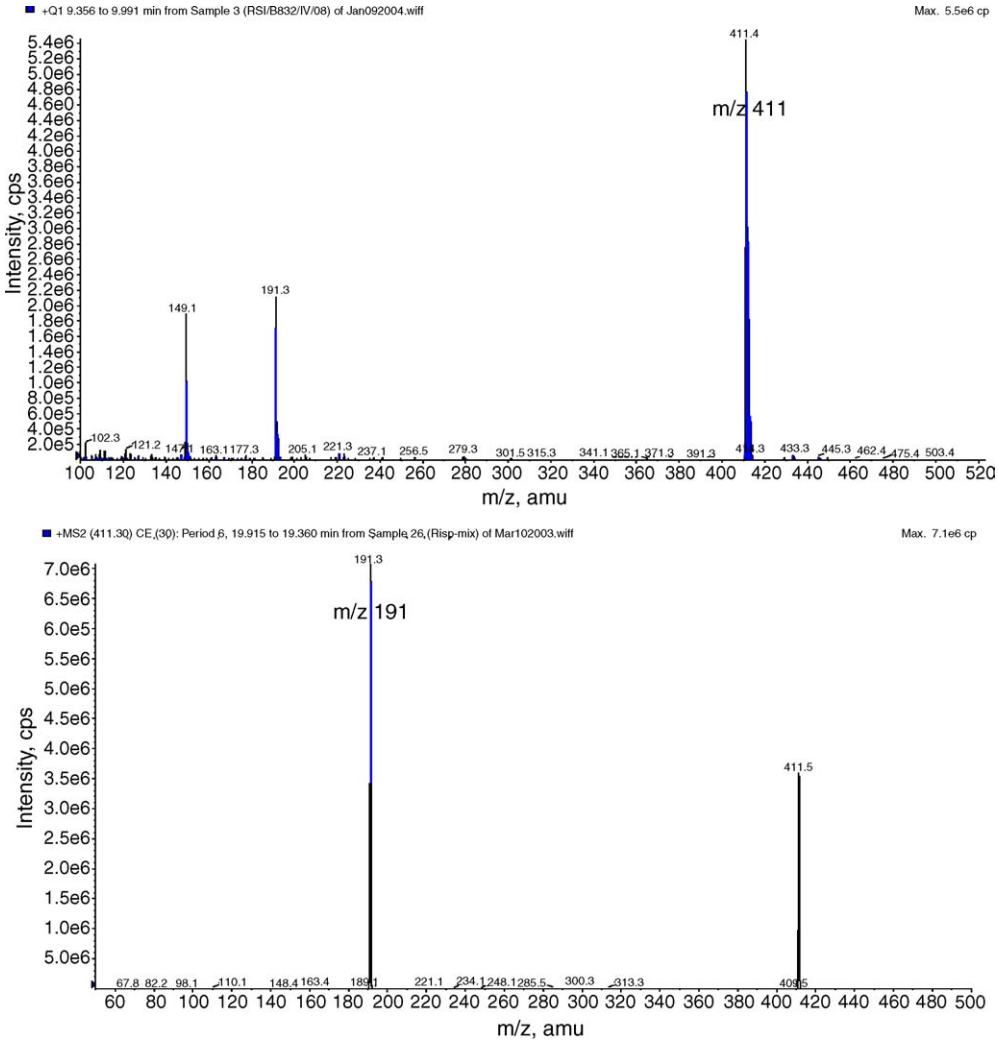

Fig. 2. MS and MS/MS of RSP.

2.3. Mass spectrometry

The LC-MS Analysis has been performed on API 3000 PE Sciex mass spectrometer. The analysis was performed in positive ionization mode with Turbo Ion Spray interface with the following conditions. Ion source voltage 5200 V, declustering potential 70 V, entrance potential 10 with the nebuliser gas as nitrogen at 8 psi. LC-MS-MS experiment is done with a CE of 60 V.

2.4. NMR studies

The ^{1}H and ^{13}C NMR studies were done at 400 and 100 MHz, respectively, in $CDCl_3$ for RSP and RSP-2. CD_3OD is used for RSP-1 since solubility in $CDCl_3$ is very less. These experiments were done on a Varian Mercury plus 400 MHz FT NMR spectrometer. The ^{1}H chemical shifts are reported on the δ scale in ppm, relative to tetra methyl silane (TMS) (δ 0.00), while ^{13}C chemical shifts relative to $CDCl_3$ (δ 77.00) and CD_3OD (δ 49.05) as internal standards. Distortionless enhancement by polarization transfer (DEPT) spectral editing revealed the presence of methyl and methine groups as positive peaks while the methylenes as negative peaks.

2.5. Single crystal XRD studies

Single crystals suitable for X-ray diffraction have been grown from chloroform and benzene. The intensity data have been collected on Rigaku AFC-7S single crystal diffractometer [4] using Mo Kα radiation (λ = 0.7107 Å) with CCD Mercury area detector. The structure has been solved by direct methods SIR92 [5] and refined using least squares procedures with the Crystal Structure software [6].

3. Results and discussion

3.1. Structure elucidation of impurities

3.1.1. Mass spectral studies

The HPLC analysis (Fig. 1) of risperidone showed a polar impurity (referred as RSP-1) and a non-polar impurity (referred as RSP-2) at 0.65 and 1.35 RRT, respectively. The molecular ion

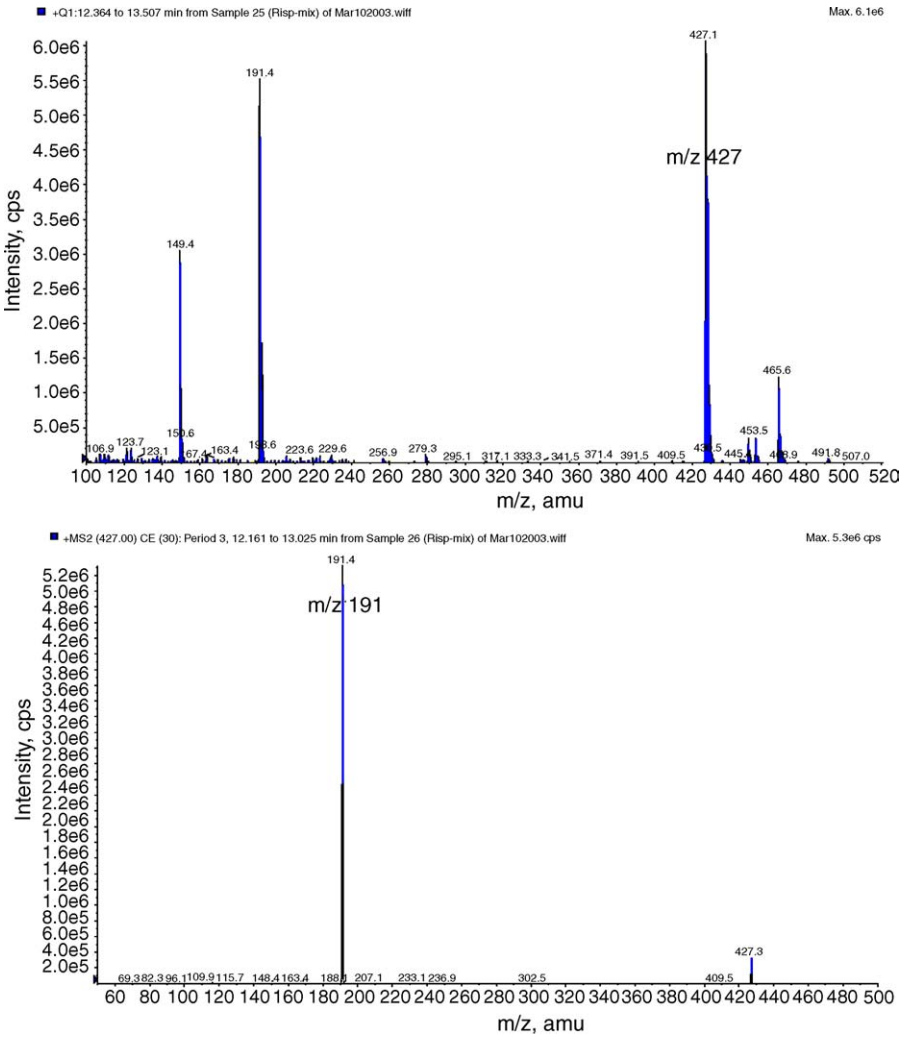

Fig. 3. MS and MS/MS of RSP-1.

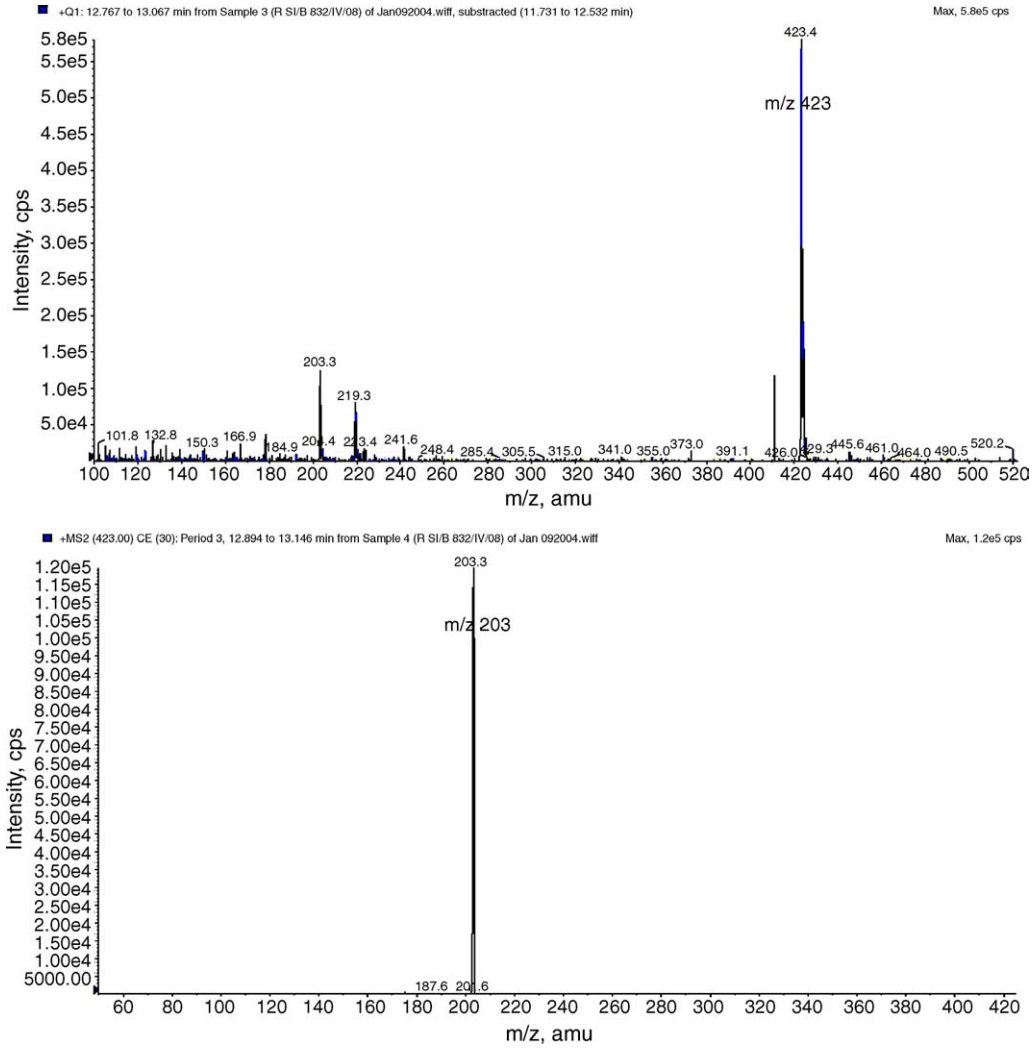

Fig. 4. MS and MS/MS of RSP-2.

information for these impurities has been obtained from online LC-MS data. The mass spectra of RSP, RSP-1 and RSP-2 are shown in Figs. 2–4. They displayed protonated molecular ions at *m*/*z* 411, 427, and 423, respectively. RSP-1 is 16 amu more than RSP while RSP-2 is 12 amu more than RSP.

The structure of risperidone can be considered to be made of the two moieties A and B (Fig. 5) with *m*/*z* 191 and 219, respectively. The MS-MS analysis has been undertaken to get insight about structural details. Interestingly, both RSP and RSP-1 displayed a dominant fragment at *m*/*z* 191 in MS-MS data (Figs. 2 and 3). This observation clearly indicates that the additional 16 amu of RSP-1 should come from the moiety B (Fig. 5). An interesting possibility could be the formation of N-oxide of RSP. On the other hand, RSP-2 (Fig. 4) displayed a dominant fragment at *m*/*z* 203 (Fig. 5), which is 12 amu more than the dominant fragment of RSP, viz. 191 (Fig. 2). This is indicative of the fact that RSP-2 could have some structural modification in the moiety A of RSP. Further structural information about the impurities could not be derived from the MS-MS data. Hence, these impurities were isolated by column chromatography and were subjected for further structural investigations.

3.2. Spectral studies of impurities

3.2.1. RSP-1

3.2.1.1. NMR studies. The ^{1}H and ^{13}C NMR spectral data of RSP-1 were compared with those of RSP in Tables 1 and 2, respectively. The numbering scheme for the NMR assignments, is shown in Fig. 5. In either data, the information about coupling due to ^{19}F observed in RSP-1 is comparable with those of RSP.

In the case of RSP-1, the number of proton and carbon resonances is same as that in RSP. However, the ^{1}H and the ^{13}C chemical shifts of the methylene groups attached to the nitrogen atom in the piperidine ring are deshielded when compared to those of RSP. This observation lends support to the hypothesis (*vide supra*) of the formation of N-Oxide in the piperidine ring. This is in well agreement with the Mass fragmentation pattern observed for risperidone-N-Oxide. The NMR experiments were done using CD_3OD solvent as the impurity is not completely soluble in $CDCl_3$.

3.2.1.2. Single crystal studies. The molecular structure of the RSP-1 is further confirmed by Single crystal XRD studies. The

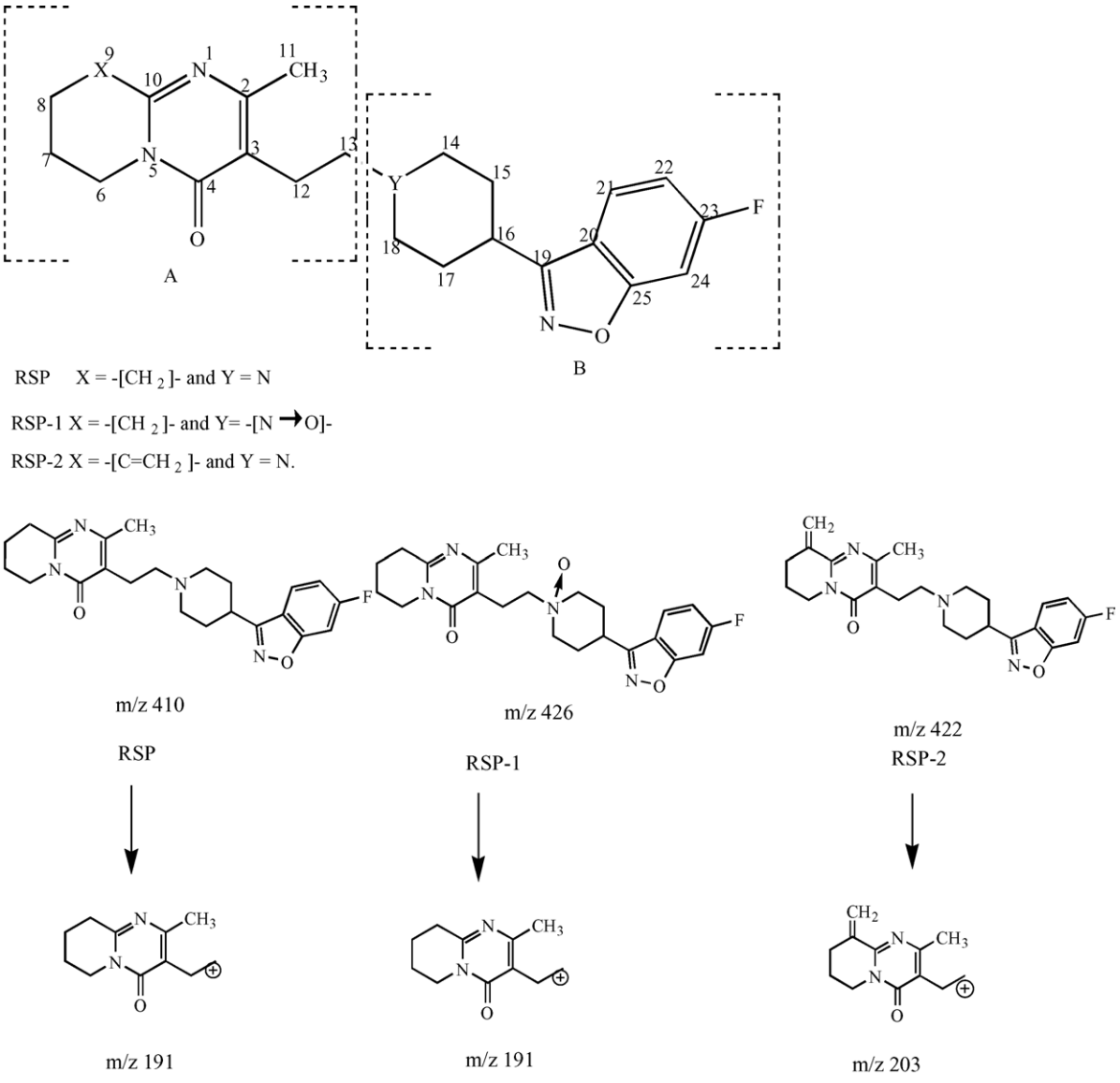

Fig. 5. Structure and Mass fragmentation of RSP, RSP-1 and RSP-2.

Table 1
^{1}H NMR assignments

Position[1]	RSP-1			RSP			RSP-2		
	^{1}H	δ (ppm)	^{2}J	^{1}H	δ (ppm)	^{2}J	^{1}H	δ (ppm)	^{2}J
6	2H	3.94	t,6.0	2H	3.95	t,5.4	2H	4.01	t,5.2
7	2H	3.45	m	2H	2.77	m	2H	2.00	m
8	2H	3.50	m	2H	2.85	m	2H	2.64	m
9	2H	2.00	–	2H		m	2H	–	–
11	3H	2.40	s	3H	2.32	s	3H	2.39	s
12	2H	1.90	m	2H	1.96	m	2H	2.80	m
13	2H	3.20	t,7.0	2H	2.55	m	2H	2.57	t,7.0
14, 18	H_a	3.70	m	H_a	3.19	m	H_a	3.19	m
	H_b	2.90		H_b	2.26		H_b	2.30	
15, 17	H_a	2.80	m	2H	2.12	m	2H	2.10	m
	H_b	2.20	m						
16	1H	3.45	m	1H	3.08	m	1H	3.10	m

1: Refer Fig. 5 for numbering; 2: This column gives the ^{1}H–^{1}H coupling constant (s) singlet, (t) triplet, (m) multiplet,.

Table 2
^{13}C NMR assignments

Position[a]	RSP-1		RSP		RSP-2	
	DEPT	δ (ppm)	DEPT	δ (ppm)	DEPT	δ (ppm)
2	–	161.02	–	157.7	–	158.59
3	–	117.34	–	116.8	–	120.23
4	–	164.43	–	161.9	–	162.84
6	CH_2	44.21	CH_2	42.1	CH_2	43.42
7	CH_2	22.66	CH_2	23.2	CH_2	21.88
8	CH_2	31.98	CH_2	30.9	CH_2	28.87
9	–	19.83	CH_2	18.7	CH_2	136.94
10	–	161.46	–	160.5	CH_2	151.33
12	CH_2	21.11	CH_2	21.4	CH_2	21.88
13	CH_2	69.34	CH_2	56.2	CH_2	56.66
14, 18	CH_2	64.45	CH_2	52.8	CH_2	53.38
15, 17	CH_2	23.23	CH_2	30.0	CH_2	30.59

[a] Refer Fig. 5 for numbering.

RSP-1 crystallizes as colorless needles in monoclinic space group $P2_1/a$ with cell dimensions $a = 17.459(2)$, $b = 7.5617(9)$, $c = 17.180(2)$ Å, $\beta = 108.06(2)^\circ$ and $V = 2155.5(5)$ Å^3 containing four molecules in the unit cell. The final R factors are: R (Rw) = 0.065(0.080) (with 3289 observed reflections). There is a water molecule in the lattice. Thus, the structure of the RSP-1 is determined to be 4-(6-fluorobenzo[d]isoxazol-3yl)-1-[2-(2-methyl-4-Oxo-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-3-yl)ethyl]hexahydro-1-pyridiminiumolate.

All the bond parameters are normal [7]. The fluoro isoxazol moiety (A) is perfectly planar with maximum in plane deviation of 0.0124 Å for C8. The piperidine ring (B) assumes a perfect chair conformation where the atoms N2 and C8 deviate by 0.6856 and −0.6997 Å, respectively from the least squares plane defined by the rest of the ring atoms. In 2-methyl-6,7,8,9-tetrahydro-4H-pyrido[1,2a] pyrimidin-4-one moiety (C) the methylene carbon atoms C21 and C22 connected, respectively to C20 and C23 are found to be disordered. Due to this disorder the two methylene carbon atoms were not refined anisotropically and no attempts were made to locate or geometrically fix their hydrogen atoms. They occupy two positions each with occupancy 0.5. This leads to two space averaged half-chair conformers. In one conformer C211 and C221 is displaced by 0.217(9) and −0.499(9) Å, respectively from the least square plane defined by the rest of the four atoms of the 6-membered ring. On the other hand, the atoms C212 and C222 deviate by −0.583(8) and 0.249(8) Å from the plane.

In the crystal structure RSP-1, the molecules are held together by hydrogen-bonding, C—H···F and C—H···O interactions. The RSP-1 crystallizes as monohydrate. The water molecule donating its two hydrogens connects the RSP-1 molecules which are related by two fold screw axis into a chain propagating along 'b' axis. Strong interaction such as C2—H1···F1 and C5—H3···O4 connect the molecules across the centers of symmetry. These interactions are shown in Table 3. The RSP-1 could be formed by the oxidation of risperidone.

Table 3
Hydrogen-bonding geometry (Å)

D—H···A	D—H	H···A	D···A	D—H···A
O4—H29···O2	0.84(4)	1.97(4)	2.797(3)	172(3)
C9—H4···O2	0.9500	2.5600	2.889(3)	100.00
O4—H28···O2	0.94(5)	1.89(5)	2.829(3)	177(2)
C2—H1···F1	0.9500	2.4900	3.438(4)	173.00
C5—H3···O4	0.9500	2.5400	3.431(4)	157.00

Symmetry codes: (i) $1-x, -1/2+y, 3/2-z$; (ii) $2-x, -y, 1-z$; (iii) $1-x, -y, 1-z$.

3.2.2. RSP-2

3.2.2.1. NMR studies. The ^{1}H and ^{13}C NMR spectral data of RSP-2 were compared with those of RSP in Tables 1 and 2, respectively. The numbering scheme for the NMR assignments is shown in Fig. 5. In either data, the information about coupling due to ^{19}F observed in RSP-2 is comparable with those of RSP.

The spectral data of RSP-2 is comparable with that of RSP but for the following differences. The signal due to the methylene group at C9 is missing. Instead, an olefinic methylene group and an additional quaternary carbon are observed. The additional 12 amu in RSP-2 than RSP can be rationalized in terms of an exocyclic double bond in moiety A of RSP at C9 position. The observed ^{1}H coupling constant for the olefinic protons is characteristic of exocyclic double bond. The long range H–C–C–C correlation observed in the HMBC experiment confirms the position of the exocyclic bond to be at C9. This structure is in well agreement with the diagnostic fragment observed in MS/MS data.

3.2.2.2. Single crystal studies. The RSP-2 crystallizes as colorless blocks in monoclinic space group P21/c with cell dimensions $a = 9.530(3)$, $b = 17.174(5)$, $c = 13.135(4)$ Å, $\beta = 94.687(7)^\circ$ and V = 2142(1)Å^3 containing four molecules in the unit cell. The final R factors are: R (Rw) = 0.034(0.040) (with 4502 observed reflections). All the bond parameters are normal. Thus the molecular structure of the RSP-2 has been determined to be 3-{2—[4-(6-fluorobenzo [d] isoxazol-3-yl) piperidino] ethyl}-2-methyl 9-methylene-6, 7, 8, 9-tetrahydro-4H-pyrido [1,2a]-Pyrimidine-4-one.

All the bond parameters are normal. The fluoro isoxazol moiety (A) is perfectly planar with maximum inplane deviation of 0.0366 Å for C8. Strong interaction between C2—H1···F1 is observed which thereby connects RSP-1 molecules across centre of symmetry. The piperidine ring(B) assumes a perfect chair conformation with C9, C10, C11 and C12 constituting the planar part whereas N2 and C8 are located 0.6932 Å and −0.6614 Å, respectively from the least squares plane defined by the rest of the ring atoms. In 2-methyl-9-methylene-6, 7, 8, 9-tetrahydro-4H-pyrido [1,2a] pyrimidin-4-one moiety(C) the carbon atom C21 is deviating from the plane by −0.6663 resulting in a Sofa conformation.

In the crystal structure RSP-1, the molecules are held together by C—H···O interactions (Table 4).

Table 4
Hydrogen-bonding geometry (Å)

D—H···A	D—H	H···A	D···A	D—H···A
C5—H2···O2	0.9500	2.3000	3.231(3)	165.00
C20—H20···O1	0.9500	2.5800	3.468(4)	155.00

Symmetry codes: (i) $-1+x, y, z$; (ii) $1+x, 1/2-y, -1/2+z$.

RSP-2 is a process related impurity and it is formed when dichloromethane is used as solvent in the first step of the synthetic scheme of risperidone.

4. Conclusions

The present study illustrates the application of online LC-MS/MS as a diagnostic tool in the identification of impurities in the analysis of risperidone. The two impurities were isolated and are characterized by mass and NMR spectral studies which are further confirmed unambiguously by single crystal XRD studies.

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