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Identification of an unknown trace-level impurity in bulk drug of Seroquel by high-performance liquid chromatography combined with mass spectrometry

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Abstract

An impurity was detected in bulk drug Seroquel at about 0.4% level by the reversed-phase high-performance liquid chromatography with UV detection. The accurate mass of impurity was measured by FTICR equipped with electrospray ionization interface, and the structure of impurity was characterized on the basis of the on-line multi-stage mass spectrometric evidences. The proposed structure was further confirmed by multi-stage mass spectrometry of Seroquel and four related compounds.

Keywords: Seroquel; Impurity; HPLC–MS/MS; Structural elucidation

1. Introduction

Typical antipsychotic neuroleptics, such as loxapine, clothiapine and haloperidol are generally potent dopamine (DA) receptor antagonists, especially at the DAD_2 receptor subtype, with high propensity to induce extrapyramidal side effects (EPS) and tardive dyskinesias (TDs) [1,2,4]. The atypical antipsychotic clozapine which does not induce TDs, is relatively free of other EPS [3]. Still, a significant obstacle to a wide use of clozapine, is the increased risk of agranulocytosis [2,5]. Seroquel, 11-(4-[2-(2-hydroxyethoxy)ethyl]piperazin-1-yl)dibenzo[*b*,*f*][1,4]thiazepine, which has similar multireceptor interaction to clozapine are efficacious and well-tolerated antipsychotic. A particular clinical advantage of Seroquel over other antipyschotics is the freedom from EPS and lack of prolactin elevation over its entire antipsychotic dose range [6–8].

The impurity profile of a drug substance is important for its safety assessment and is critical to monitor its manufacturing process. Therefore, it is important to test the impurities for regulatory requirements. The International Conference on Harmonization (ICH) guidelines specify that any component of a pharmaceutical product which is not the chemical entity of active substance or excipient, present at levels higher than 0.1% or 1 mg/day intake (whichever is lower) for a maximum daily dose of 2 g/day or less, need to be identified and qualified with appropriate toxicological studies. For a daily dose of greater than 2 g of drug substance, the identification threshold is 0.05% [9].

Due to the fact that the impurities are usually process-related compounds, they are most probably structurally similar to the synthesized target drugs. High-performance liquid chromatography (HPLC), as a highly efficient separation technique in combination with mass spectrometry is particularly useful for rapid and accurate characterization of structurally related compounds, instead of complex and time-consuming preparative separation and purification [10–12]. As well-known, the main advantage of the Fourier transform ion cyclotron resonance (FTICR) is the ability to perform accurate mass measurement, not only of the production ion that can deduce elemental composition of molecules, but also of fragment ions gained by MS/MS experiment that giving an additional dimension to MS/MS data [13]. In this work, we describe the identification of an unknown trace-level impurity detected in the bulk drug Seroquel by HPLC combined with mass spectrometry.

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2. Experimental details

2.1. Materials

The samples of Seroquel and three related compounds 1, 2, 3, 4 were obtained from Huihai Inc. (Taizhou, China). Analytical grade ammonium formate and formic acid were bought form Beijing Xudong Chemical Co. (Beijing, China) and Yixing Second Chemical Reagent Co. (Yixing, Jiangshu, China), respectively. HPLC grade acetonitrile and methanol were both bought from Merck Co. (Darmstadt, Germany) and water was purified by a Milli-Q purification system (Millipore, Bedform, USA).

2.2. Analytical high-performance liquid chromatography (HPLC)

Analytical HPLC was performed on an Agilent 1100 series HPLC equipped with a G1312A binary pump, a G1314A variable wave detector (VWD). A model 7725 injection valve fitted with a 20 μl sample loop was used, along with an Agilent Chemstation date system. The separation was achieved on a Sciencehome C18 column (4.6 mm × 250 mm, 5 μm). The column was kept at 40 °C. The mobile phase consisted of a mixture of acetonitrile (90%, v/v) and (10%, v/v) aqueous buffer. The aqueous buffer was prepared by dissolving 10 mM ammonium formate in purified water adjusted with formic acid to pH 3.0. The flow rate was set at 1.0 ml/min and the effluent was monitored at 240 nm.

2.3. Mass spectrometry

HPLC/ESI-MS^n analyses were carried out on the Agilent HPLC system described above combined with a Bruker Esquire 3000^{plus} ion trap mass spectrometer (Bruker-Franzen Analytik GmbH, Bremen, Germany) equipped with an electrospray ionization source. Instrument control and data acquisition were performed using Esquire 5.0 software. The samples were infused into the source chamber from the HPLC system with a T-junction introducing a splitting ratio of 2:1. The ion source temperature was 250 °C and the ESI needle voltage was always set at 4.0 kV. Nitrogen was used as drying gas at a flow rate of 10 ml/min and the nebulizer gas at a back-pressure of 30 psi. Both the on-line tandem mass spectra were obtained by collision-induced dissociation (CID) with helium as collision gas after isolation of precursor ions and optimization of the collision energy between 0.5 and 1.0 V to maximize the ion current in the spectra.

The off-line accurate mass spectrometric experiments were performed on an Apex III 7.0 T FTICR mass spectrometer (Bruker, Daltonic, Billerica, MA, USA) combined with an Apollo ESI source operated in the positive ion mode. Solution introduction was accomplished using a Cole-Parmer syringe pump at a rate of 3.0 μl/min. Accurate mass measurements were performed using NaI as an external calibrant. MS^n analysis was performed through isolation of the desired precursor ion using a correlated sweep. Sustained off-resonance excitation (SORI) was applied for collision-induced dissociation (CID). The collision gas pulsed into the ICR cell was argon. Each spectrum is an average of four transients, each composed of 512 K points, acquired using a SGI workstation operating XMASS version 6.1.1.

2.4. Nuclear magnetic resonance spectrometer

NMR spectra were recorded with a Bruker Advance DMX 500 instrument with a QNP probe head at ambient temperature. The data was acquired on Silicons Graphics O2 workstations using XWINNMR version 2.1 (Bruker Analytik, GmbH, Germany).

3. Results and discussion

3.1. HPLC/MS analysis

During the routine impurity profiling of the bulk Seroquel drug substance by analytic HPLC, a potential impurity at a level of over 0.4% was detected. A typical HPLC chromatogram using the method as described in the experimental details was shown (see Fig. 1). The positive ESIMS spectra displayed the protonated molecular ion peak at *m/z* 505 ($[M+H]^+$) for the impurity and *m/z* 384 for Seroquel, indicating that the impurity might have the molecular mass of 504. The off-line ESI-FTIC-MS spectra not only confirmed this result (see Table 1), but also provided the information about the elemental composition of molecule of the impurity. The molecular formula for the impurity obtained by high-resolution ESI-FTICR-MS measurement was $C_{30}H_{24}N_4S_2$.

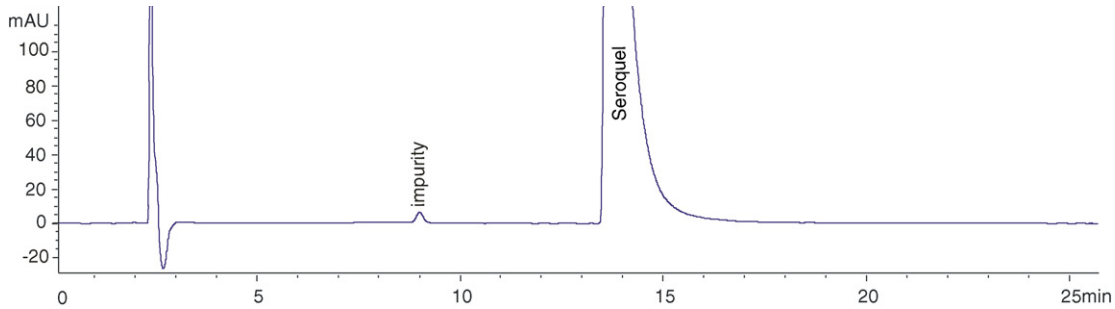


Fig. 1. HPLC chromatogram of the bulk Seroquel.

Table 1
Measured accurate mass results obtained for the impurity

Compound	Measured accurate mass of precursor ion	Calculated monoisotopic mass of precursor ion	Measurement error (ppm)	Deduced elemental formula of molecule
Impurity	505.1504	505.1515	2.2	$C_{30}H_{25}N_4S_2^+$
	527.1318	527.1335	3.2	$C_{30}H_{24}N_4S_2Na^+$

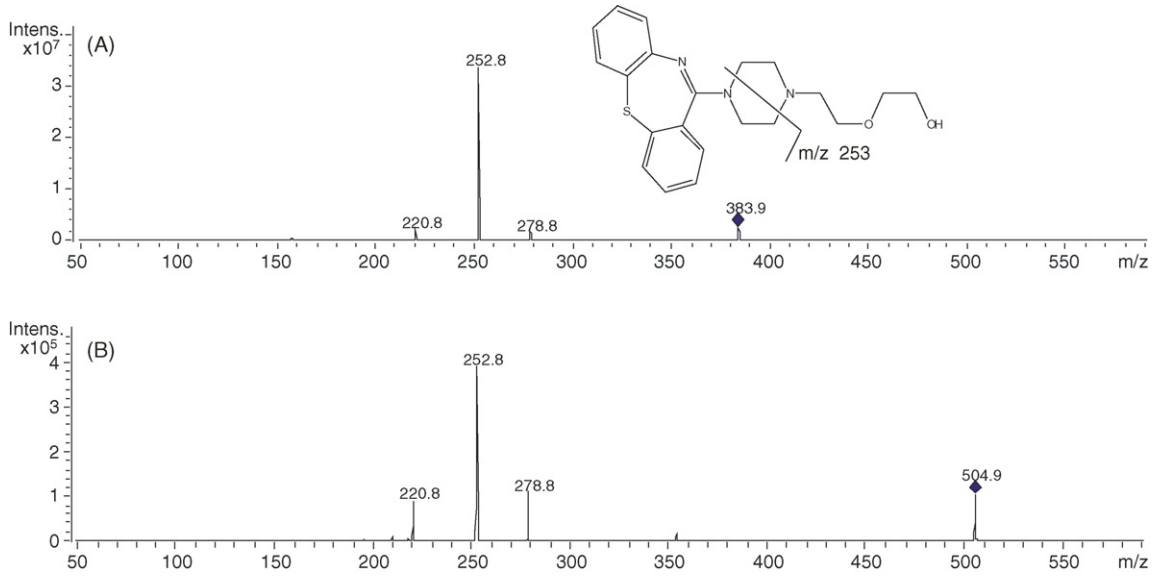
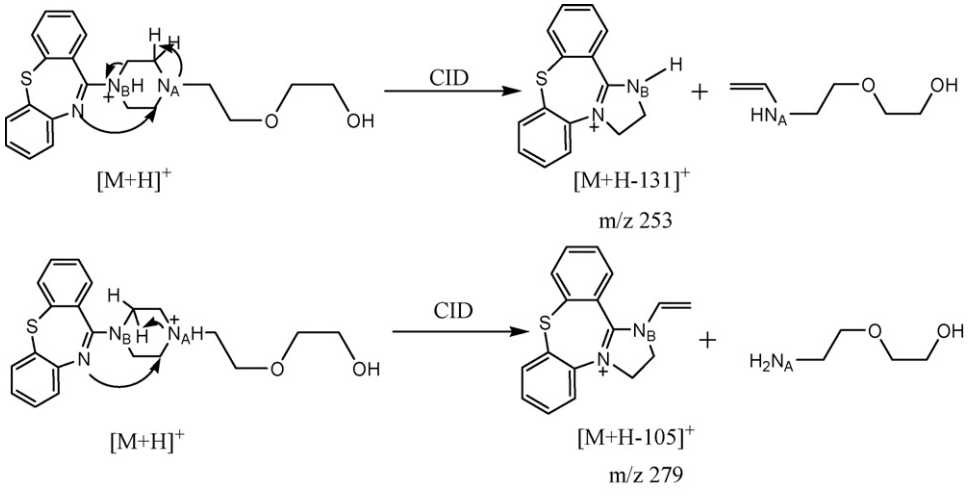


Fig. 2. (A) ESI/MS^2 spectrum of protonated Seroquel (CE = 0.65 V) and (B) ESI/MS^2 spectrum of protonated impurity (CE = 0.70 V).

3.2. HPLC/MS^n analysis

The product ion spectrum of protonated Seroquel is reported in Fig. 2(A), and the cleavage of the piperazine ring takes place relatively easily, which is similar in appearance to that obtained by Jari et al. [13]. The dissociation productions at *m/z* 253 and *m/z* 279 were produced by the proposed fragmentation pathways that are shown in Scheme 1. Their accurate mass were 253.0770 (consistent with $C_{15}H_{13}N_2S^+$) and 279.0921 (consistent with $C_{17}H_{15}N_2S^+$) obtained by the high-resolution ESI-FTICR-MS/MS, respectively. The lowest energy fragmentation was the loss of 131 Da from the $[M+H]^+$ ion, which requires extensive rearrangement in combination with the cleavage of the piperazine ring. The formation of the $[M+H-105]^+$ ion can be rationalized in terms of the breaking of two C–N_A bonds instead of C–N_A and C–N_B bonds of the piperazine ring.

The ESIMS/MS spectrum of protonated impurity is shown in Fig. 2(B), and the impurity has the same dissociation productions at *m/z* 253 and *m/z* 279 with the Seroquel. To further investigate the structural relationship between the same fragments at *m/z*



Scheme 1. Proposed pathways of the dissociation productions at *m/z* 253 and *m/z* 279.

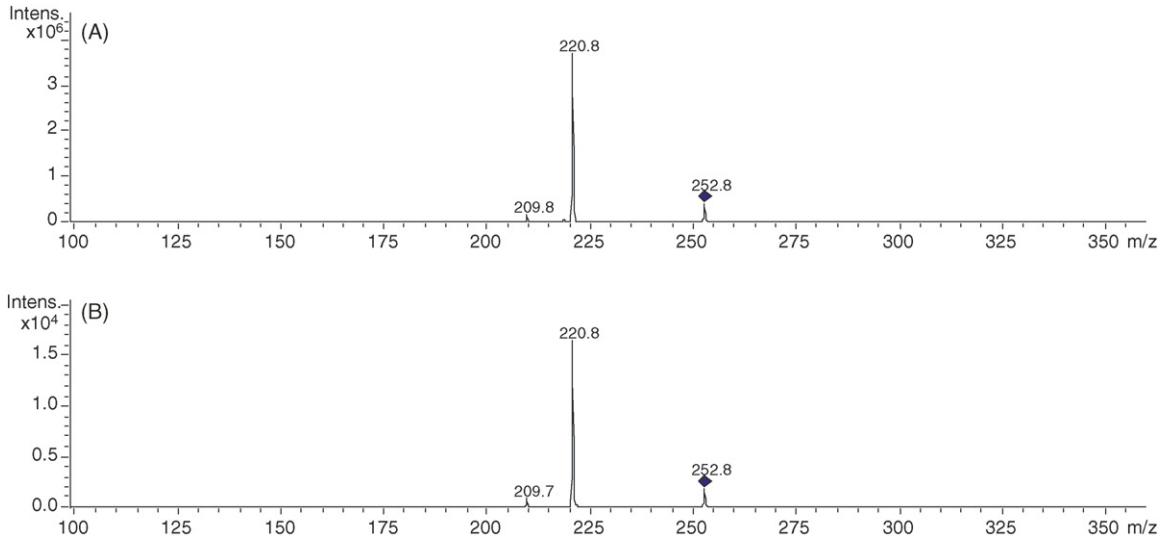


Fig. 3. (A) ESI/MS^3 spectrum of *m/z* 253 derived from protonated Seroquel and (B) ESI/MS3 spectrum of *m/z* 253 derived from protonated impurity (CE = 0.75).

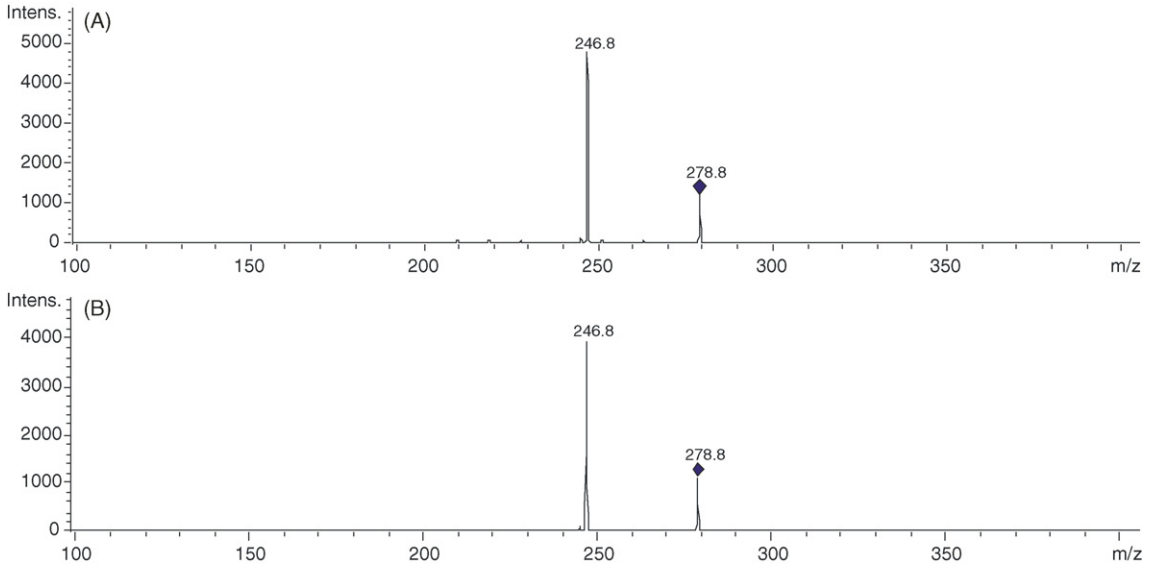


Fig. 4. (A) ESI/MS^3 spectrum of *m/z* 279 derived from protonated Seroquel and (B) ESI/MS^3 spectrum of *m/z* 279 derived from protonated impurity (CE = 0.78).

Table 2
Summary of sequential fragmentation spectra for seroquel in positive ion mode

Sequential fragmentation summary (CE)	*m/z* Value (relative abundance)			
$384^+ \rightarrow$ frags (0.65 V)	221 (7)	253 (100)	279 (5)	384(8)
$384^+ \rightarrow 279^+ \rightarrow$ frags (0.75 V)	247 (100)	279 (25)	–	–
$384^+ \rightarrow 253^+ \rightarrow$ frags (0.78 V)	210 (4)	221 (100)	253 (11)	–

Table 3
Summary of sequential fragmentation spectra for compound 1 in positive ion mode

Sequential fragmentation summary (CE)	*m/z* Value (relative abundance)					
$296^+ \rightarrow$ frags (0.75 V)	210 (5)	221 (11)	227 (21)	253 (100)	279 (6)	296(34)
$296^+ \rightarrow 279^+ \rightarrow$ frags (0.72 V)	247 (58)	279 (100)	–	–	–	–
$296^+ \rightarrow 253^+ \rightarrow$ frags (0.73 V)	210 (3)	221 (100)	253 (44)	–	–	–

Table 4
Summary of sequential fragmentation spectra for compound 2 in positive ion mode

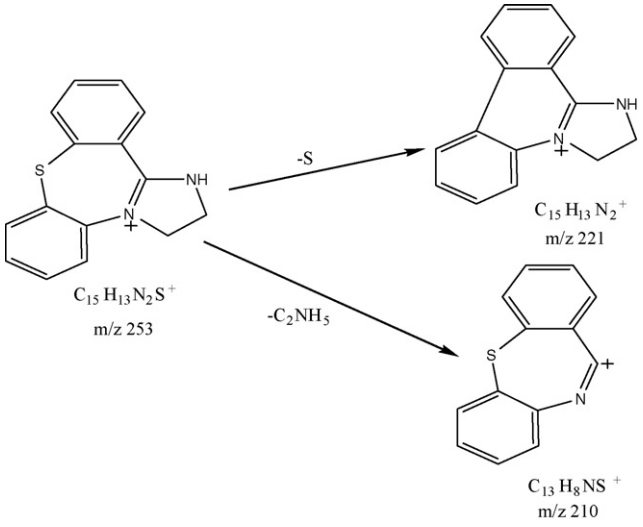
Sequential fragmentation summary (CE)	*m/z* Value (relative abundance)			
$428^+ \rightarrow$ frags (0.65 V)	221 (8)	253 (100)	279 (6)	428(14)
$428^+ \rightarrow 279^+ \rightarrow$ frags (0.83 V)	247 (100)	279 (29)	–	–
$428^+ \rightarrow 253^+ \rightarrow$ frags (0.73 V)	210 (2)	221 (100)	253 (44)	–

Table 5
Summary of sequential fragmentation spectra for compound 3 in positive ion mode

Sequential fragmentation summary (CE)	*m/z* Value (relative abundance)		
$328^+ \rightarrow$ frags (0.75 V)	271 (100)	297 (1)	328 (25)

Table 6
Summary of sequential fragmentation spectra for compound 4 in positive ion mode

Sequential fragmentation summary (CE)	*m/z* Value (relative abundance)		
$327^+ \rightarrow$ frags (0.75 V)	270 (100)	296 (1)	328 (31)



Scheme 2. Proposed pathways of fragmentations at *m/z* 221 and 210 from the dissociation product ions at *m/z* 253.

253 and *m/z* 279, MS^3 experiments on the fragments of *m/z* 253 and *m/z* 279 derived from the protonated Seroquel and the protonated impurity were carried out for comparison. Interestingly, the two dissociation spectra of the fragments with *m/z* 253 derived from different precursor ion (see Fig. 3(A and B)) exhibit a high degree of similarity. So are the two dissociation spectra of the fragment with *m/z* 279 (see Fig. 4(A and B)). Both of fragmentations at *m/z* 279 showed a facile loss of atom S to give the production ion at *m/z* 247 and no other significant fragmentation channels were observed. Both of the ions at *m/z* 253 showed a loss of 43 Da to give the production ion at *m/z* 210, besides, a easy loss of atom S to produce ions at *m/z* 221. Loss of S usually are diagnostic of sulfur heterocycles [14]. The dissociation products at *m/z* 221 and *m/z* 247 are very stable, because both of them cannot dissociate into small pieces even if the CID energy is higher than 1.1 V in the MS^4 experiment. This observation could be rationalized by the formation of a very stable conjugated system (see Scheme 2). These results suggested that the structures of the two dissociation ions at *m/z* 253 were the same. So are the structures of the two dissociation ions at *m/z* 279. It also means that the structure of impurity (see Fig. 5(A)) has the same structural unit with Seroquel, and only different from −R group (−R of Seroquel stands for $-CH_2CH_2OCH_2CH_2OH$).

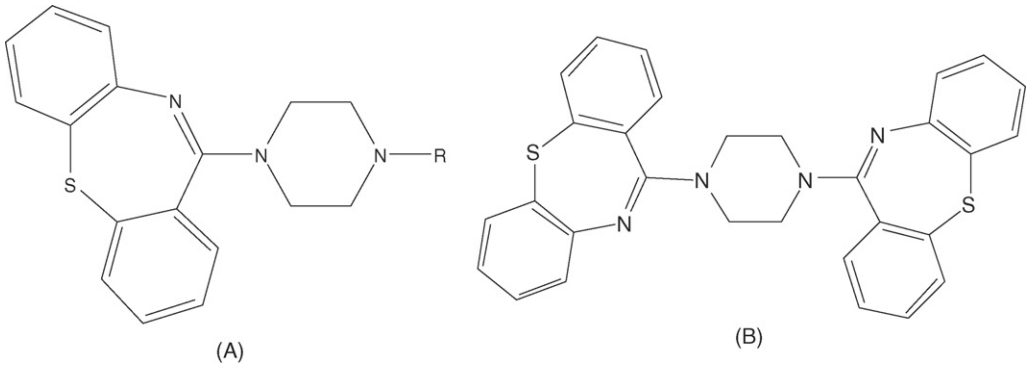
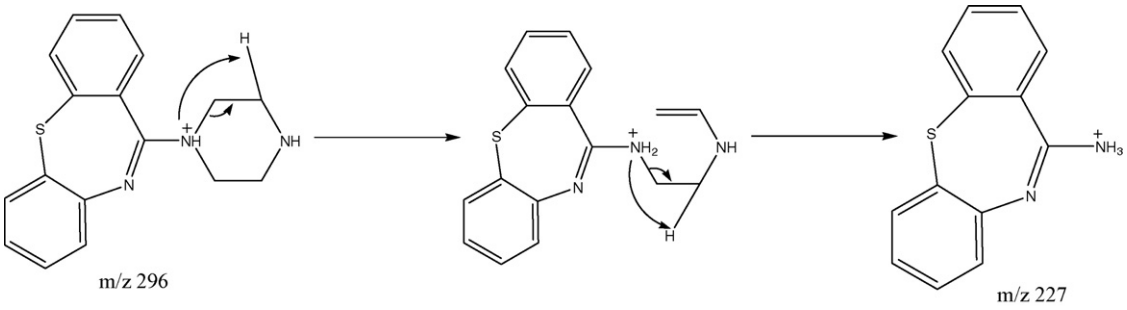
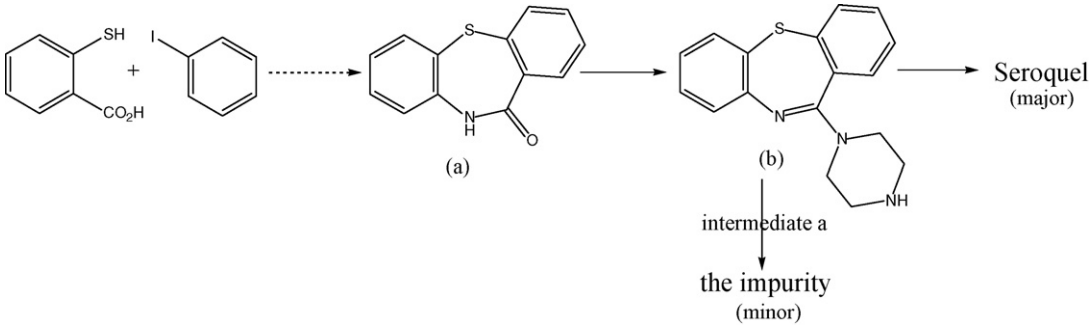


Fig. 5. Proposed structure of the impurity of interest.



Scheme 3. Proposed pathways of fragmentations at *m/z* 227 from the protonated analog A.



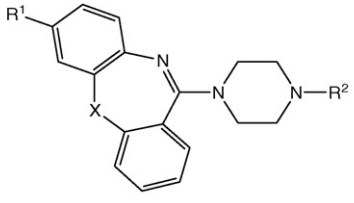
Scheme 4. Proposed formation pathway for the impurity.

In order to further support the proposed structure (see Fig. 5(A)), multi-step fragmentation of Seroquel and four analogs 1, 2, 3, 4 (see Fig. 6) were investigated and the results are displayed in Tables 2–6. It can be seen that all the five compounds underwent losses of CH_2=CH–NHR^2 and H_2NR^2 in their MS^2 experiments. The Seroquel and two analogs 1 and 2, with the same groups of R^1, X and with different groups of $-R^2$, all produced the fragment ions at *m/z* 253 and 279. Additionally, analog 1, whose piperazine ring group is not substituted, produced the fragmentation at *m/z* 227, with an accurate mass value of 227.0638 Da (consistent with $C_{13}H_{11}N_2S^+$) obtained by off-line FTICR-MS/MS. Loss of 67 Da from protonated analog 1 was observed as a significant fragmentation channel (see Scheme 3) which requires two times of hydrogen rearrangement in combination with the cleavage of the piperazine ring. The analogs 3 and 4 with different groups of –X and $-R^2$ groups also easily cleavage the piperazine ring, and resulted in the fragmentation of $[M+H-NHR^2]^+$ and $[M+H-C_2H_4NR^2]^+$.

As mentioned above, the elemental composition of the impurity was $C_{30}H_{24}N_4S_2$, and the known structural part of the impurity was $C_{17}H_{16}N_3S$. So the elemental composition of the unknown –R group of the impurity was $C_{13}H_8NS$. Obviously, it is the same with the elemental composition for the fragmentation at *m/z* 210 that produced from the fragmentation at *m/z* 253. Even more, knowing details on synthetical procedure of Seroquel [7] enabled proposal of the structure shown in Fig. 5(B). Minor intermediate 'b' reacted with the intermediate 'a' to the impurity (see Scheme 4). To our knowledge, this is a novel impurity, which may be the by-product resulting from the synthesis of the bulk drug Seroquel.

3.3. NMR analysis

The structure of impurity was confirmed by ^{1}H NMR and ^{13}C NMR experiments after preparative isolation. Selected data: ^{1}H NMR (500 MHz, CF_3COOD): δ 7.63–7.56 (m, 2H), δ 7.48–7.34 (m, 8H), δ 7.25–7.14 (m, 6H), δ 4.49 (m, 1H), δ 4.43 (m, 1H), δ 4.26–4.20 (m, 2H), δ 3.94 (m, 1H), δ 3.60 (m, 1H), δ 3.57 (m, 1H); ^{13}C NMR (500 MHz, CF_3COOD): δ 169.19, δ 168.68, δ 168.51, δ 145.23, δ 45.10, δ 139.23, δ 138.98, δ 138.82, δ 138.62, δ 137.70, δ 137.04, δ 136.98, δ 134.24, δ 134.02, δ 133.50, δ 133.40, δ 133.33, δ 131.30, δ 131.16, δ 128.92, δ 128.72, δ 54.12, δ 50.50, δ 49.64, δ 48.68.



Seroquel	X=S	R^1=H	R^2=$CH_2CH_2OCH_2CH_2OH$
A	X=S	R^1=H	R^2=H
B	X=S	R^1=H	R^2= $(CH_2CH_2O)_2CH_2CH_2OH$
C	X=N	R^1=Cl	R^2=CH3
D	X=O	R^1=Cl	R^2=CH3

Fig. 6. Structures of Seroquel and four analogs.

4. Conclusions

A rapid HPLC/MS method was developed for the identification of an unknown trace-level impurity detected in the bulk drug Seroquel. By comparison of the multi-stage mass spectrometry result for Seroquel and the impurity (positive ESI/MS^n) and based on accurate mass data obtained by FTICR, the structure was proposed as shown in Fig. 5(B). Multi-stage CID fragmentation of Seroquel and four related compounds firmly supported this conclusion.

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