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Short communication

Identification of oxidative degradation impurities of Olanzapine drug substance as well as drug product

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ABSTRACT

Impurities found in stressed and stability studies of Olanzapine (polymorphic form-I) [1–7] in both drug substance and drug product are described. These impurities are identified as 4-(4-methyl-1-piperazinyl)-3-hydroxymethylidene-1*H*-benzo[b][1,4]diazepine-2(3H)-thione (hydroxymethylidene thione) and (Z)-4-(4-methyl-1-piperazinyl)-3-acetoxymethylidene-1*H*-benzo[b][1,4]diazapine-2(3H)-thione (acetoxymethylidene thione). An oxidative degradation pathway of Olanzapine, for the formation of these impurities, has been proposed.

1. Introduction

Olanzapine, chemically known as 2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-b][1,5]benzodiazepine is an antipsychotic drug [1–7]. It is an antagonist of dopamine D-1 and D-2 receptors, in addition has antimuscarinic anticholinergic properties and antagonist activity at noradrenergic with α-receptors. It is useful in treating in psychotic conditions. Several impurities related to oxidative degradation of Olanzapine have been reported in literature and pharmacopoeia [8–12] (Fig. 1). Baertschi et al. reported thiolactam and lactam as oxidative impurities (Fig. 1) of the drug product which are observed on storage and exposure to thermal stress at 40–60 °C. Hiriyanna et al. reported the oxidative degradation impurities A, B and C of Olanzapine drug substance (Fig. 1). During the stability and stress studies of Olanzapine polymorph form-I in both drug substance and drug product, we have found two unknown impurities related to oxidative degradation, hydroxymethylidene thione and acetoxymethylidene thione. Over the decades, as per regulatory requirement and ICH guide lines [13] it is mandatory that, any new impurities present in the drug substance and drug product above the threshold limit need to be identified and characterized.

2. Experimental

2.1. Materials and reagents

Olanzapine (polymorph form-I) drug substance, drug product (prepared from Olanzapine polymorph form-I) and samples enriched with these impurities were obtained from our laboratory. HPLC grade methanol, HPLC grade acetonitrile, AR grade sodium hydroxide, AR grade ethylenediaminetetraacetic acid disodium salt dihydrate and AR grade orthophosphoric acid (85%, v/v) were obtained from Merck India. Sodium dodecyl sulphate obtained from CDH. Milli-Q water obtained from water purification system, Elix Millipore, India, was used.

2.2. High performance liquid chromatography (analytical)

Waters HPLC system equipped with Alliance 2695 series low pressure quaternary gradient pump along with photo diode array detector and auto sampler has been used for the analysis of samples. The data was collected and processed using Waters "Empower 2" software. A Superspher® 60 RP-8, 4 μ (250 × 4.0 mm) (Merck) column was employed for the separation of impurities from Olanzapine. The column eluent was monitored at 220 nm. The sample diluent was a mixture of 0.05 M ethylenediaminetetraacetic acid disodium salt dihydrate of pH 3.0 adjusted with orthophosphoric acid and acetonitrile in the ratio of 6:4 (v/v), filter through 0.45 μ or finer porosity membrane filter.

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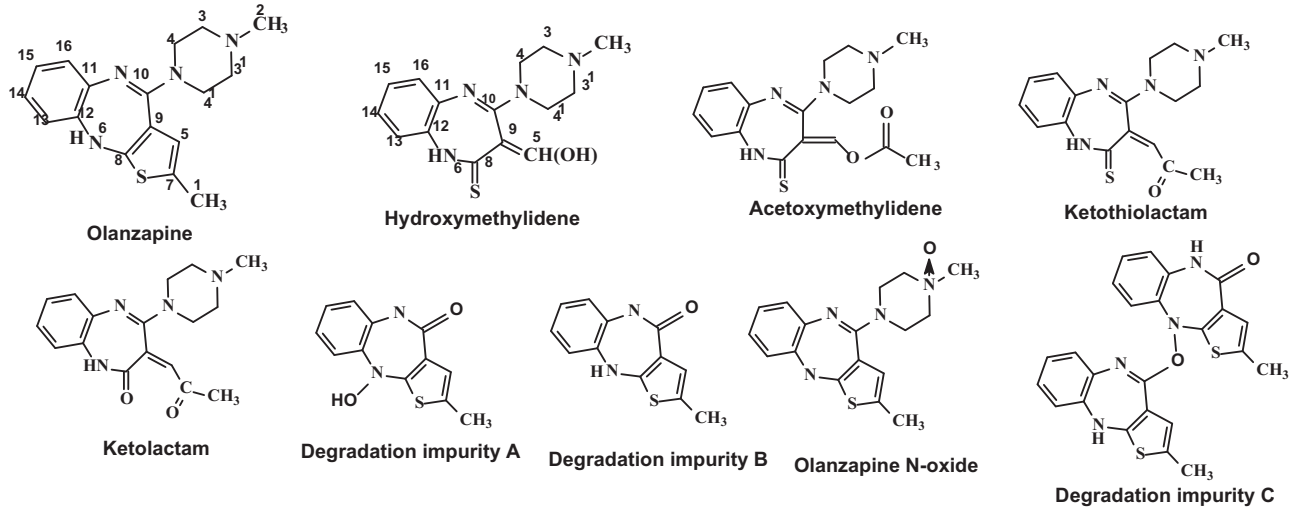

Fig. 1. Olanzapine and its oxidative degradation impurities.

2.2.1. Drug related substances HPLC method

A simple gradient reverse-phase HPLC method was optimized for the separation of impurities where the mobile phase A and B are 52:48 and 30:70 (v/v) 30 mM sodiumdodecylsulphate, 50 mM orthophosphoric acid (pH adjusted to 3.0 with sodium hydroxide)/acetonitrile, respectively. HPLC method for drug substance: the solvent composition was held at 100% mobile phase A for 10 min then increased mobile phase B linearly from 0 to 100% for 30 min and held at 100% B for 10 min. The chromatographic run time was 60 min.

HPLC method for drug product (tablet): the solvent composition was held at 100% mobile phase A for 10 min then increased mobile phase B linearly from 0 to 30% for 20 min and held at 30% B for 20 min, then increased linearly from 30 to 100% for 5 min and held at 100% B for 5 min. The chromatographic run time was 60 min.

2.3. High performance liquid chromatography (preparative)

A Waters preparative HPLC system equipped with waters liquid controller pump, photo diode array detector, auto sampler fitted with 5000 μL loop was used. The data was collected and processed using Waters empower software. An inertsil C18 column (250 × 20 mm, 5-Micron) was employed for loading the sample. An analytical method was developed in isocratic mode separately to resolve these impurities, followed by scaling up the same method for prep-HPLC to collect the required impurity fractions. The mobile phase consisted of 2 mmol/L ammonium acetate and acetonitrile in the ratio of 60:40 (v/v). The flow rate was set at 25 mL/min. Detection was carried out at 220 nm. Approximately 500 mg/mL of sample was prepared using a sample diluent. The sample diluent is a mixture of 0.05 M ethylenediaminetetraacetic acid disodium salt dihydrate of pH 3.0 adjusted with orthophosphoric acid and acetonitrile in the ratio of 6:4 (v/v).

2.4. Mass spectrometry (LC/MS/MS)

Initial LC/MS/MS analysis has been performed on SCIEX-API 2000, Mass Spectrometer (Applied Biosystems). The analysis was performed in positive ionization mode with turbo ion spray interface. The parameters for ion source voltage IS = 5500 V, declustering potential, DP = 70 V, focusing potential, FP = 400 V, entrance potential, EP = 10 V were set with nebuliser gas as air at a pressure of 40 psi and curtain gas as nitrogen at a pressure of 25 psi in API 2000 mass spectrometer. Further to get accurate mass, analysis was performed on high resolution mass spectrometer using electro spray ionization Q-TOF SYNAPT (Waters India). The accurate mass obtained from the instrument, theoretical mass and mass error was mentioned in Table 1. An Inertsil ODS-3V (250 × 4.6 mm, 5-Micron, GL Sciences, Japan) column was used for the separation. The mobile phase was a mixture of 2 mmol/L ammonium acetate and acetonitrile in a ratio of 60:40 (v/v). The analysis was performed at a flow rate of 1.0 mL/min with splitting.

2.5. NMR spectroscopy

The ^{1}H, ^{13}C NMR and DEPT experiments were carried out for unknown impurity hydroxymethylidene thione at processional frequencies 300.1315 MHz and 75.1365 MHz, in DMSO-d_6 at 25 °C on a Bruker Avance-300 FT NMR spectrometer. ^{1}H and ^{13}C chemical shifts are recorded on the δ scale in ppm, relative to tetra methyl silane (TMS) d 0.00 and DMSO-d_6 at 39.5 ppm in ^{13}C NMR respectively.

2.6. Detection of impurities by HPLC

Typical HPLC chromatogram of Olanzapine and its impurities observed in drug substance as well as in drug product obtained by using the HPLC method (Section 2.2 and Fig. 2). The observed impurities under study are, hydroxymethylidene thione and acetoxymethylidene thione, eluted at retention times of about 2.8 min and 7.2 min respectively, while Olanzapine eluted at about 19 min in drug substance HPLC method, but in drug product these impurities are eluted at retention times of about 4 min and 15 min respectively, while Olanzapine eluted at about 40 min.

2.7. Isolation of hydroxymethylidene thione impurity by prep HPLC

A simple reverse phase chromatographic system, discussed under Section 2.2 was used for isolating the unknown impurity hydroxymethylidene thione. In this chromatographic system, the hydroxymethylidene thione impurity eluted at about 5.4 min. So fractions eluting between 5.2 and 5.8 min were collected, pooled and concentrated by evaporating acetonitrile at room temperature under high vacuum on a Buchii Rotavapour Model R124. The aqueous layer was extracted with methylene chloride. Methylene chloride layer was evaporated at room temperature under vacuum to obtained hydroxymethylidene thione impurity using Buchii

Table 1
Mass fragmentation of Olanzapine and the impurity mass *m/z* 344.

Name of the compound	Mass observed in LCMS/Q-TOF system	Theoretical mass	Mass error (ppm)	Molecular formula
Olanzapine	313.1485	313.1487	0.6	$C_{17}H_{21}N_4S$
Fragment 1	282.1067	282.1065	0.7	$C_{16}H_{16}N_3S$
Fragment 2	256.0907	256.0908	0.4	$C_{14}H_{14}N_3S$
Fragment 3	213.0484	213.0486	0.9	$C_{12}H_9N_2S$
Acetoxymethylidene impurity	345.1383	345.1385	0.6	$C_{17}H_{21}N_4O_2S$
Hydroxymethylidene impurity	303.1279	303.1280	0.3	$C_{15}H_{19}N_4OS$
Fragment 1	246.0698	246.0696	0.8	$C_{12}H_{12}N_3OS$
Fragment 2	269.1399	269.1402	1.1%	$C_{15}H_{17}N_4O$

Rotavapour Model R124. Purity was checked by HPLC, which was found to be ~95%, and was characterized by NMR, mass experiments. Attempts were made to isolate another unknown impurity acetoxymethylidene thione, but we noticed that this impurity is unstable and converting into hydroxymethylidene thione impurity under the conditions used.

2.8. Formulation of tablet

The Olanzapine tablet was prepared using Olanzapine (polymorph form-I) Active Pharma Ingredient. The excipients used in the tablet formulation are Lactose monohydrate, Crospovidone, Hydroxy propyl cellulose Magnesium stearate and Purified water. The tablets are manufactured using wet formulation process.

3. Results and discussion

Olanzapine (polymorph form-I) drug substance and Olanzapine tablets (prepared from Olanzapine polymorph form-I, immediate release and orally disintegrating tablets) were subjected to stability as per ICH guidelines. The 3 months 40 °C/75% RH accelerated stability samples showed two unknown impurities eluting at RRT about 0.13 and about 0.33 in drug related substances method and at RRT about 0.11 and 0.41 in tablet related substances method (Fig. 2). The UV absorbance spectrum of these unknown impurities are different from Olanzapine (Fig. 4). The LC–MS analysis showed the *m/z* value for these unknown impurities as 303 $[M+H]^+$ and 345 $[M+H]^+$ respectively in both HPLC methods. Attempts were made to isolate impurity of *m/z* 345 $[M+H]^+$ by preparative HPLC. It was observed that the impurity is unstable in basic, acidic also in neutral buffer and was converted into unknown impurity of *m/z* 303 $[M+H]^+$. To further investigate the chemical structure of the unknown impurity, Olanzapine (polymorph form-I) drug substance sample was kept at 105 °C for six days wherein impurity with *m/z* 344 got enriched to 0.37% and *m/z* 302 increased to 0.90%. This sample was subjected to LCMS/ESI Q-TOF. The high resolution mass analysis using Mass Lynx fragmentation tool, proposed the following two probable elemental compositions/molecular formulae with a mass error of 0.6 ppm: (i) $C_{17}H_{21}N_4O_2S$, (ii) $C_{16}H_{25}O_6S$. The second formula of these two elemental compositions, was ruled out due to absence of nitrogen atom. This is due to the fact that this impurity is being derived from Olanzapine molecule, which contains four nitrogen atoms. Based on the high resolution mass fragmentation study in comparison to the reported fragmentation pattern of

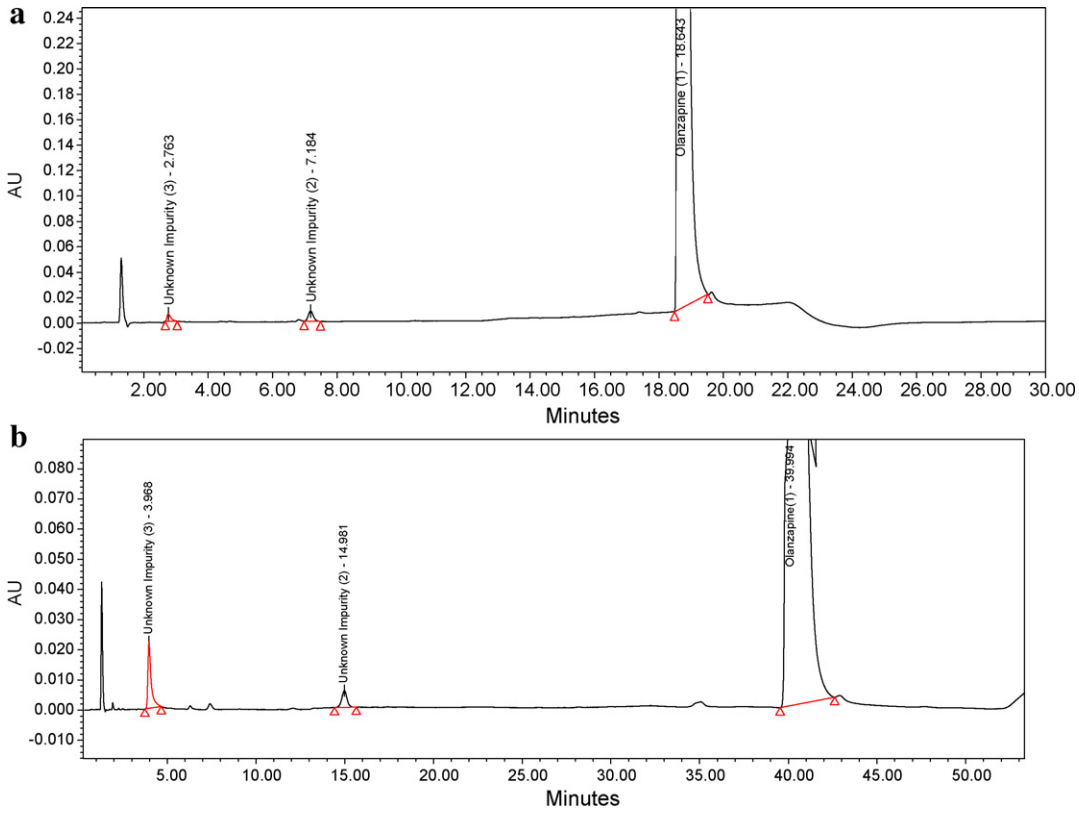

Fig. 2. A typical analytical HPLC chromatogram of Olanzapine and its impurities, (a) drug substance using drug substance related substances method and (b) drug product using drug product (tablet) related substances method.

Table 2
^{1}H NMR and ^{13}C NMR assignments for Olanzapine and hydroxymethylidene thione impurity.

Position	Olanzapine		Hydroxymethylidene thione	
	^{1}H (ppm)/^{1}H/multiplicity	^{13}C (ppm)	^{1}H (ppm)/^{1}H/multiplicity	^{13}C (ppm)
1	2.20/3H/s	15.7	–	–
2	2.26/3H/s	46.4	2.32/3H/s	44.84
3,3′	2.38/4H/m	55.1	2.79–2.87/4H/m	53.94
4,4′	3.32/4H/m	47.1	3.70–4.02/4H/m	48.4
5	6.63/1H/s	123.2	8.97/1H/brs	170.1
6	7.58/1H/brs	–	9.26/1H/brs	–
7	–	128.7	–	–
8	–	154.0	–	188.2
9	–	118.8	–	117.5
10	–	158.1	–	164.2
11	–	141.3	–	139.7
12	–	144.6	–	140.3
13, 14, 15, 16 (aromatic)	6.67–6.84/4H/m	119.5, 123.2, 123.9, 124.1	6.91–7.23/4H/m	120.9, 121.3, 124.0, 125.1
17	–	–	6.55/1H/s	–

Note: For numbering refer Fig. 1; s = singlet; m = multiplet; brs = broad signal.

Olanzapine, the chemical structure of the unknown impurity of *m*/*z* 344, assigned as acetoxymethylidene thione impurity. It is also further supported by the fact that it contains a liable acetoxy group, which gets hydrolyzed to produce hydroxymethylidene thione with *m*/*z* 302. The observed LCMS Q-TOF fragments of Olanzapine and the impurity *m*/*z* 344 are shown in Table 1.

Subsequently, ^{1}H and ^{13}C NMR spectra of the isolated compound of unknown impurity hydroxymethylidene thione compared with that of Olanzapine was described in Table 2. The NMR data indicate the presence of a 1,2-disubstituted benzodiazepine and the substituted N-methyl piperazine structure intact when compared with corresponding Olanzapine ^{1}H and ^{13}C chemical shifts. The only significant difference in the ^{1}H and ^{13}C NMR spectra is that chemical shift due to thiophen ring is absent. The chemical shift due to $-CH_3$ of position '1′ is not observed in the isolated compound hydroxymethylidene thione impurity in both ^{1}H and ^{13}C NMR spectra. The ^{13}C signal was not observed for position '7′ and one additional broad signal observed at 6.55 ppm in the ^{1}H NMR spectrum which is exchanged by D_2O. Based on the above high resolution mass spectral data and NMR data, it is proposed that the unknown impurity is 4-(4-methyl-1-piperazinyl)-3-hydroxymethylidene-1*H*-benzo[b][1,4] diazepine-2(3H)-thione (hydroxymethylidene thione) (Fig. 1).

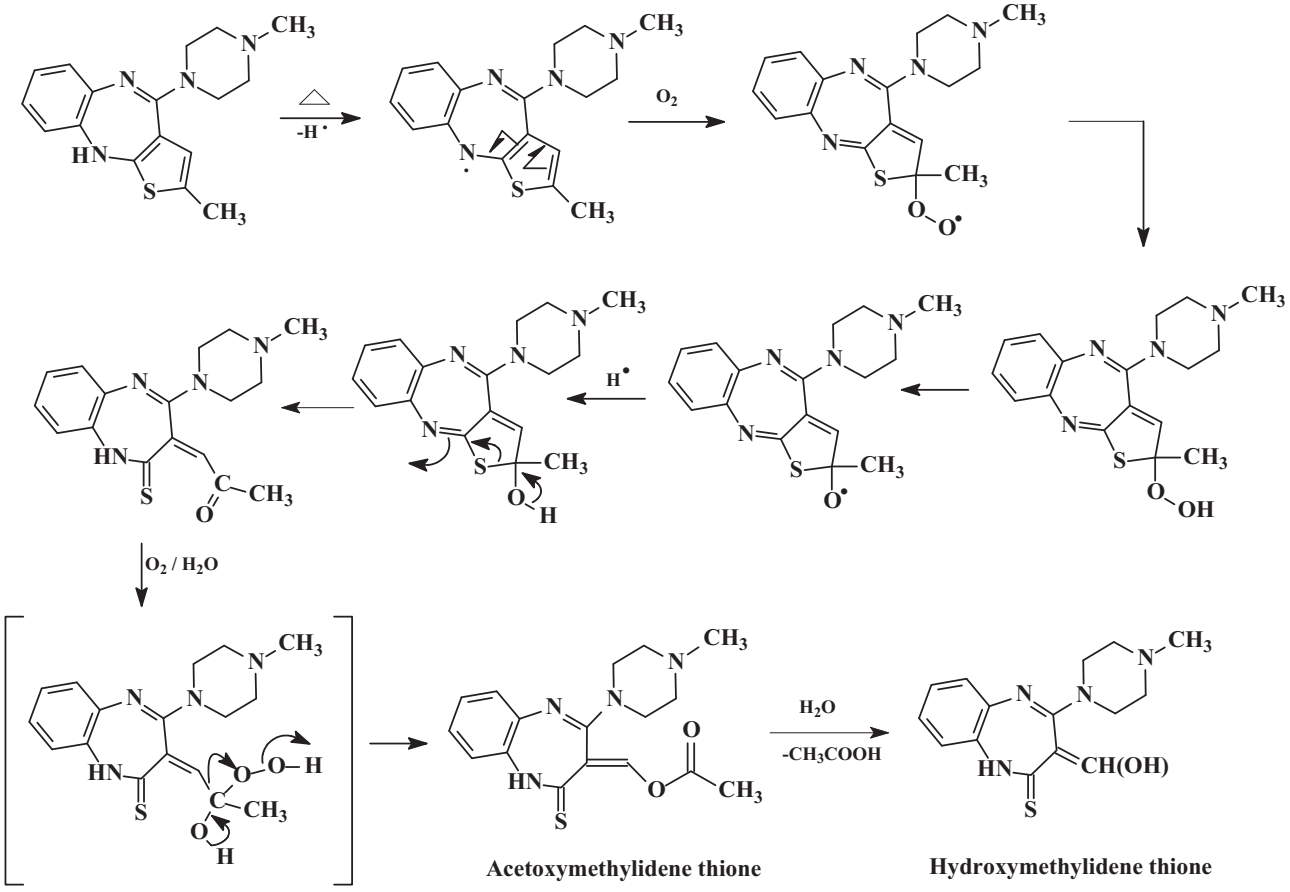

Fig. 3. The degradation pathway of the impurities hydroxymethylidene thione and acetoxymethylidene thione.

Table 3
Degradation profile of Olanzapine drug substance (form-I and form-II) at 105 °C.

	Olanzapine form-I			Olanzapine form-II		
	Initial	3 days	6 days	Initial	3 days	6 days
Hydroxymethylidene	Not detected	0.28%	0.9%	Not detected	Not detected	Not detected
Acetoxymethylidene	Not detected	0.27%	0.575	Not detected	Not detected	Not detected

We believe that more precisely the thiophen ring of Olanzapine was very much prone to degradation in the presence of oxygen. The possible mechanism for the formation of these impurities is shown in Fig. 3. We also prepared Olanzapine polymorphic form-II in laboratory, and subjected Olanzapine polymorphic form-I and form-II to stress studies by exposing the sample in open petri dish at 105 °C for six days in an oven. The impurities, hydroxymethylidene thione and acetoxymethylidene thione are formed up to ~1% and ~0.5% respectively in the Olanzapine polymorphic form-I whereas these impurities are not observed in Olanzapine polymorphic form-II in HPLC analysis, results are shown in Table 3. We have carried out thermal stress studies of Olanzapine (drug substance) form-I and form-II, in the dark as well as in the presence of light and we have found no differences in the results of impurity pattern.

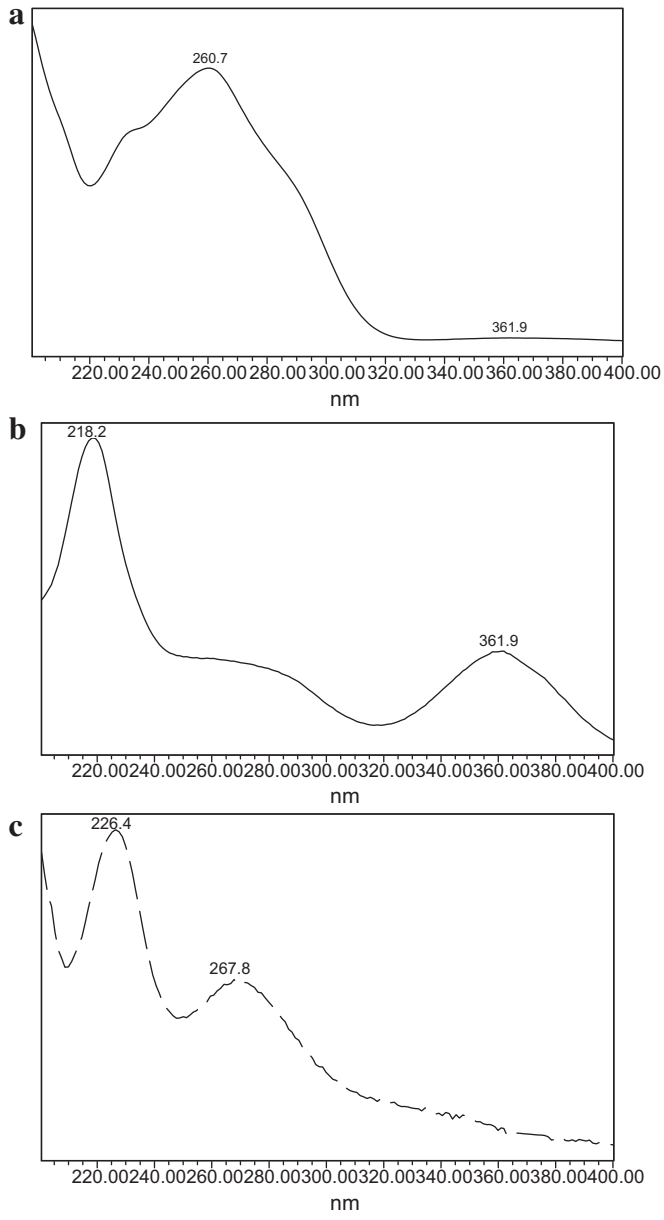

Fig. 4. UV spectra of (a) Olanzapine, (b) Olanzapine impurity *m*/*z* 302 and (c) Olanzapine impurity *m*/*z* 344.

To investigate, role of oxygen for this degradation [14], we carried out stability studies of drug substance (polymorph form-I) by keeping oxygen scavenger in the stability pack at condition of 40 °C/75% RH for 3 months and 6 months. The level of these unknown impurities are not detected in drug substance packed with oxygen scavenger as against 0.83% and 0.12% obtained at RRT about 0.13 and about 0.33 respectively in packs without using oxygen scavengers. The accelerated stability data of Olanzapine immediate release tablets and orally disintegrating tablets (prepared from Olanzapine drug substance, polymorphic form-I) clearly reveal that Olanzapine acetoxymethylidene thione impurity is formed initially by action of heat and air. In presence of moisture this impurity further gets hydrolysed to Olanzapine hydroxymethylidene thione following a pseudo first order kinetic reaction, the rate limiting step being the availability of moisture. We could observe the levels of Olanzapine acetoxymethylidene thione and hydroxymethylidene thione impurity less than 0.5%, which are the qualification threshold levels based on the total daily intake for Olanzapine drug product as per ICH Q3 (B).

4. Conclusion

As outlived by ICH guidelines, identification, isolation of impurities is very important task during drug synthesis and storage. It can provide crucial toxicology and safety data of finished drug and dosage forms. We have identified two impurities in aged and stressed samples of Olanzapine drug substance and drug product. Those are characterized as hydroxymethylidene thione and acetoxymethylidene thione by analytical data. The results indicate that the two impurities are from degradation of Olanzapine resulting from oxidation and ring-opening of the thiophene ring. Formation of these two degradation products in stressed and aged solid-state formulations is believed to occur from auto oxidation processes catalyzed by formulation excipients.

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