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Impurity profile study of zaleplon

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Abstract

Zaleplon is a pyrazolopyrimidine derivative and possesses sedative and hypnotic properties. Seven unknown impurities in zaleplon bulk drug at levels below 0.1% were detected by reverse-phase high performance liquid chromatography (HPLC). The starting material, 3-amino-4-cyanopyrazole and an intermediate, *N*-[3-[3-(dimethylamino)-1-oxo-2-propenyl]-phenyl]-*N*-ethylacetamide (DOPEA) were also present in the sample at a level below 0.1%. The molecular weights of impurities were determined by LC-MS analysis. These impurities were isolated from crude samples of zaleplon using reverse-phase preparative HPLC. Based on the spectral data the structures of these impurities were characterized as, *N*-(3-(3-(4-amino-2H-pyrazolo [3,4-d]pyrimidin-6-yl) pyrazolo[1,5-a] pyrimidin-7-yl)phenyl)-*N*-ethylacetamide (impurity I); *N*-[3-(3-carboxamidopyrazolo[1,5-a]pyrimidin-7-yl)phenyl]-*N*-ethylacetamide (impurity II); *N*-[3-(3-cyanopyrazolo[1,5-a]pyrimidin-7-yl)phenyl]acetamide (impurity III); *N*-[3-(3-cyanopyrazolo [1,5-a]pyrimidin-7-yl)phenyl]-*N*-methylacetamide (impurity IV); *N*-[3-(3-cyanopyrazolo[1,5-a] pyrimidin-5-yl)phenyl]-*N*-ethylacetamide (impurity V); *N*-[3-(3-cyanopyrazolo[1,5-a] pyrimidin-7-yl)phenyl]-*N*-ethylamine (impurity VI); *N*-[3-(3-cyano-6-[(E)-3-((*N*-ethyl-*N*-acetyl)amino)phenyl-3-oxoprop-1-enyl] pyrazolo[1,5-a]pyrimidin-7-yl) phenyl]-*N*-ethylacetamide (impurity VII). Structural elucidation of all impurities by spectral data (^{1}H NMR, ^{13}C NMR, MS and IR) and formation of these impurities are discussed in detail.

Keywords: Zaleplon; Impurities; Isolation; Preparative HPLC; Characterization

1. Introduction

Zaleplon (*N*-[3-(3-cyanopyrazolo[1,5-a]pyrimidin-7-yl)phenyl]-*N*-ethylacetamide) is a short-acting hypnotic agent. Its molecular formula is $C_{17}H_{15}N_5O$ and molecular weight is 305.33 amu.

Many methods were reported in the literature dealing with the determination of zaleplon by HPLC. These are determination of zaleplon in human plasma by RP-HPLC with fluorescence detection [1], pharmacokinetic application [2], separation and identification of zaleplon metabolites in human urine using CE with laser-induced fluorescence detection and LC-MS [3], development and validation of HPLC-ESI-MS [4] and HPLC-APCI-MS [5] assay for the determination of zaleplon in human plasma, determination by LC-turbo-ionspray-MS: application to forensic cases [6], and assay of zaleplon tablet formulation [7]. HPLC methods in Refs. [2,7] are isocratic with ammonium acetate buffer. HPLC method described in this paper is aimed for the separation of all impurities with gradient mode and by using phosphate buffer enabling to resolve nine impurities present in zaleplon.

The HPLC analysis of zaleplon bulk drug revealed the presence of nine impurities, which were up to 0.1%. As per the regulatory requirements, the impurity profile study has to be carried out for any final product [8]. Synthesis of zaleplon and its regioisomer together with their characterization is described in the literature [9,10]. This paper describes for the first time the identification of impurities present in zaleplon, detection of molecular masses by LC-MS, isolation by preparative HPLC and characterization of impurities using spectral data.

2. Experimental

2.1. Samples

The investigated sample, zaleplon was synthesized in APL Research Centre (A unit of Aurobindo Pharma Limited,

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Hyderabad, India). All impurities were isolated from crude samples by preparative HPLC. Reagents used for analysis, i.e., ammonium acetate (GR grade), potassium dihydrogen orthophosphate (AR grade), orthophosphoric acid (~85%, w/w, AR grade), acetonitrile (HPLC grade) were procured from Merck (India) Limited. Water used was Milli-Q grade.

2.2. High performance liquid chromatography

A Waters 2695 separation module equipped with 2996 diode array detector with Empower pro data handling system [Waters corporation, MILFORD, MA 01757, USA] was used. The analysis was carried out on YMC Pack-C8, 150 mm long, 4.6 mm i.d., and 5 μm particle diameter column. Mobile phase A was phosphate buffer (pH 3.0), prepared by dissolving 1.36 g of potassium dihydrogen orthophosphate in 1000 ml of water, pH adjusted to 3.0 ± 0.05 with orthophosphoric acid. Mobile phase B was acetonitrile. UV detection was carried out at 225 nm and flow rate was kept at 1.5 ml/min. Data acquisition time was 25 min. Pump mode was gradient and the program was as follows, Time (min)/A (v/v):B (v/v); $T_{0.01}$/80:20, $T_{15.0}$/60:40, $T_{25.0}$/45:55, $T_{26.0}$/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 10 μm particle size was employed for isolation of impurities. The mobile phase consisted of (A) 0.1 M ammonium acetate solution and (B) acetonitrile for impurities I, III, IV and VII; mobile phase (A) 0.1 M ammonium acetate solution, pH adjusted to 5.0 with glacial acetic acid and (B) acetonitrile was used for impurities II and V; mobile phase (A) 0.2% glacial acetic acid and (B) acetonitrile was used for impurity VI. Flow rate was kept at 30 ml/min and UV detection was carried out at 225 nm. The gradient program was as follows, Time (min)/A (v/v):B (v/v); $T_{0.01}$/98:2, $T_{20.0}$/90:10, $T_{35.0}$/80:20, $T_{50.0}$/70:30, $T_{60.0}$/60:40, $T_{75.0}$/50:50.

2.4. LC-MS/MS analysis

LC-MS/MS analysis was carried out using Perkin-Elmer triple quadrupole mass spectrometer (API 2000, PE SCIEX) coupled with a Shimadzu HPLC equipped with SPD 10 AT VP UV–vis detector and LC 10 AT VP pumps. Analyst software was used for data acquisition and data processing. The turbo ion spray voltage was maintained at 5.5 kv and temperature was set at 375 °C. The auxiliary gas and curtain gas used was high pure Nitrogen. Zero air was used as nebulizer gas. LC-MS spectra were acquired from *m/z* 100–1000 in 0.1 amu steps with 2.0 s dwell time. Zaleplon crude laboratory batch sample was subjected to LC-MS/MS analysis. The analysis was carried out using Hypersil BDS C8, 150 mm × 4.6 mm column with 5 μm particle size. Mobile phase consisted of (A) 0.01 M ammonium acetate and (B) 1:1 mixture of acetonitrile and methanol. UV detection was carried out at 225 nm and flow rate was kept at 1.0 ml/min. Methanol was used as diluent. Data acquisition time was 40 min. Concentration of sample was 1.5 mg/ml. The gradient program was as follows, Time (min)/A (v/v):B (v/v); $T_{0.01}$/30:70, $T_{10.0}$/30:70, $T_{20.0}$/70:30, $T_{30.0}$/70:30, $T_{31.0}$/30:70. Nine peaks were detected in this sample. The masses of detected peaks were 109[$(MH)^+$], 414[$(MH)^+$], 261[$(MH)^+$], 324[$(MH)^+$], 278[$(MH)^+$], 292[$(MH)^+$], 306[$(MH)^+$] (zaleplon), 306[$(MH)^+$], 521[$(MH)^+$], 264[$(MH)^+$], respectively. From mass values the structures given in Table 1 were suggested.

2.5. NMR spectroscopy

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

2.6. Mass spectrometry

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

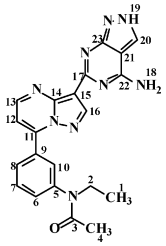
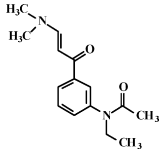
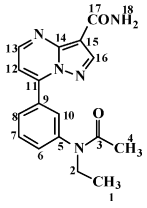
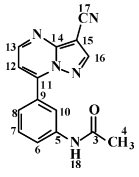
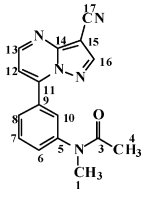
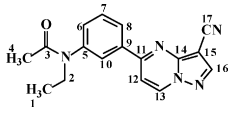
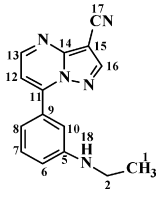
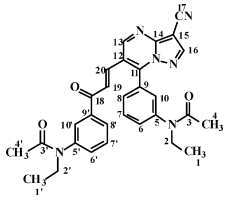
2.7. FT-IR spectroscopy

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

2.8. Isolation of impurities by preparative HPLC

Impurities were present in the crude samples at percentage levels of 0.50 (impurity I), 0.10 (impurity II), 0.17 (impurity III), 0.10 (impurity IV), 2.40 (impurity V), 0.75 (impurity VI) and 014 (impurity VII) by area normalization. All impurities were isolated by preparative HPLC from crude samples by using the conditions described in Section 2.3. Fractions collected were analyzed by analytical HPLC as per the conditions described in Section 2.2. Fractions of >90% were pooled together, concentrated on rotavapour to remove acetonitrile. Impurity V was precipitated as an off-white powder after removal of acetonitrile. It was filtered and dried at 40 °C for 2 h. In case of other impurities concentrated fractions were passed through the preparative column by using water: acetonitrile (50:50) as mobile phase to remove the buffers used for isolation. Again the eluate was concentrated using rotavapour to remove acetonitrile. The aqueous solutions were lyophilized using freeze dryer (Virtis advantage 2XL). Impurities I and VI were obtained as yellow powders and impurities II, III, IV and VII as pale yellow powders. The chromatographic purities of all impurities were determined by the HPLC method described in Section 2.2. The purities were 96.5%, 97.1%, 98.7%, 99.4%, 99.6%, 98.2% and 90.7%, respectively. Percentage yield of impurities obtained after isolation were 0.25, 0.10, 0.10, 0.075, 1.5, 0.40 and 0.10.

Table 1
Chemical structures of impurities

S. no.	RRT	Compound	Molecular weight	Structure
1	0.13	3-Amino-4-cyanopyrazole	108	
2	0.45	Impurity I	413	
3	0.63	DOPEA (intermediate)	260	
4	0.66	Impurity II	323	
5	0.72	Impurity III	277	
6	0.80	Impurity IV	291	
7	1.11	Impurity V	305	
8	1.16	Impurity VI	263	
9	1.52	Impurity VII	520	

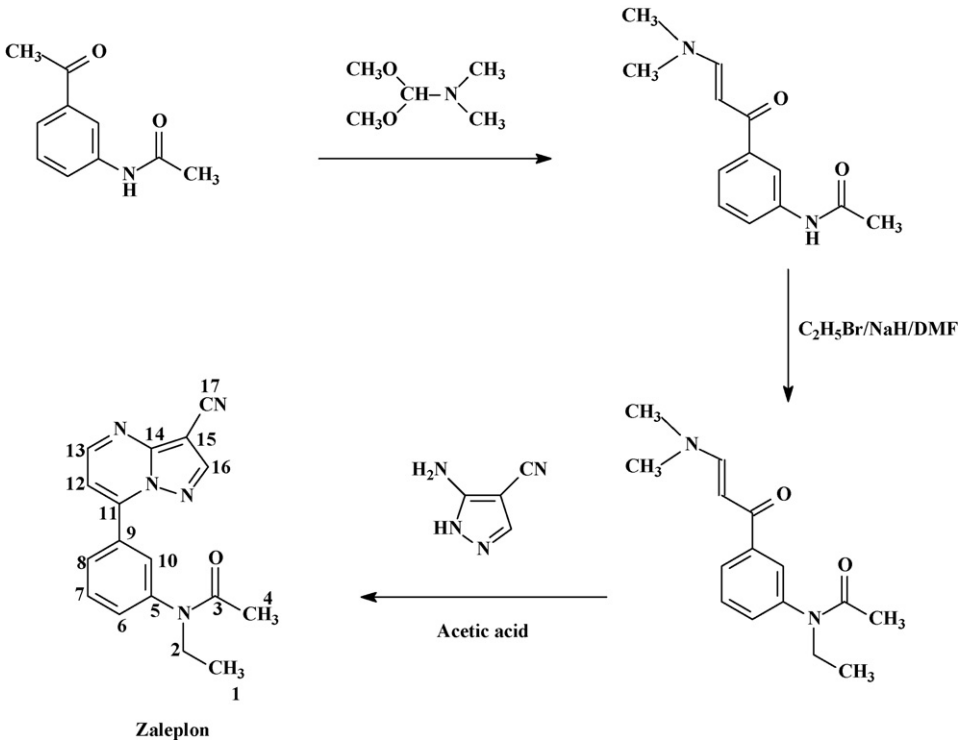


Fig. 1. Scheme for the synthesis of zaleplon.

2.9. Melting point determination

Melting points of impurities were determined on Polmon (MP96) melting point apparatus. DSC thermograms were also recorded and melting endotherms observed by DSC are comparable with the melting range values obtained by melting point apparatus.

2.10. Synthesis of zaleplon

The scheme for the synthesis of zaleplon is shown in Fig. 1.

3. Results and discussion

3.1. Detection of impurities

A typical analytical LC-chromatogram of a laboratory batch of zaleplon bulk drug is recorded using the LC method described in Section 2.2. This sample was subjected to LC-MS/MS analysis using the method described in Section 2.4. The prepared and isolated impurities were co-injected with zaleplon to confirm the retention times. All the impurities were well resolved from zaleplon peak. The resolution mixture chromatogram is shown in Fig. 2. Relative retention times of the impurities with respect to zaleplon and the structures are shown in Table 1. Spectral data and melting range are reported in the literature [9,10] for zaleplon and regioisomer, which are comparable with the observed values. HPLC method reported [9] involves gradient elution with ammonium formate buffer and RP-18 HPLC column. Unknown impurities studied in this paper were not reported except the regioisomer.

3.2. Structural elucidation of impurities

3.2.1. Impurity I

The ESI mass spectrum of impurity I (RRT-0.45) exhibited a molecular ion at *m/z*, 414 [$(MH)^+$] in positive ion mode, indicating molecular weight of 413 which was higher by 108 amu than that of zaleplon. The ^{1}H NMR spectrum showed all signals corresponding to zaleplon with small shift in the chemical shift values. In addition to that three signals were observed at 7.56 ppm, 8.08 ppm and 13.39 ppm. Signal at 7.56 ppm was broad and exchangeable with two protons integration, corresponds to NH_2 group. In ^{13}C NMR spectrum three additional quaternary carbons were observed compared to zaleplon which were absent in the DEPT spectrum. In addition to this observation, the characteristic absorption band of C≡N group at 2233 cm^{-1} was absent in the FT-IR spectrum of impurity I. Based on the above spectral data, the molecular formula of this impurity was confirmed as $C_{21}H_{19}N_9O$ and the corresponding structure was characterised as *N*-(3-(3-(4-amino-2H-pyrazolo [3,4-d] pyrimidin-6-yl)pyrazolo[1,5-a]pyrimidin-7-yl)phenyl)-*N*-ethylacetamide.

3.2.2. Impurity II

The electrospray ionization mass spectrum of impurity II (RRT-0.57) exhibited molecular ion peak at *m/z* 324 [$(MH)^+$] in positive ion mode, indicating the molecular weight of impurity as 323 which is 18 amu more than that of zaleplon. In ^{1}H NMR spectrum an extra signal was observed at 7.57 ppm with two protons integration which is broad and exchangeable and corresponds to amide protons. In IR absorption spectrum, the characteristic C≡N absorption band at 2233 cm^{-1} was

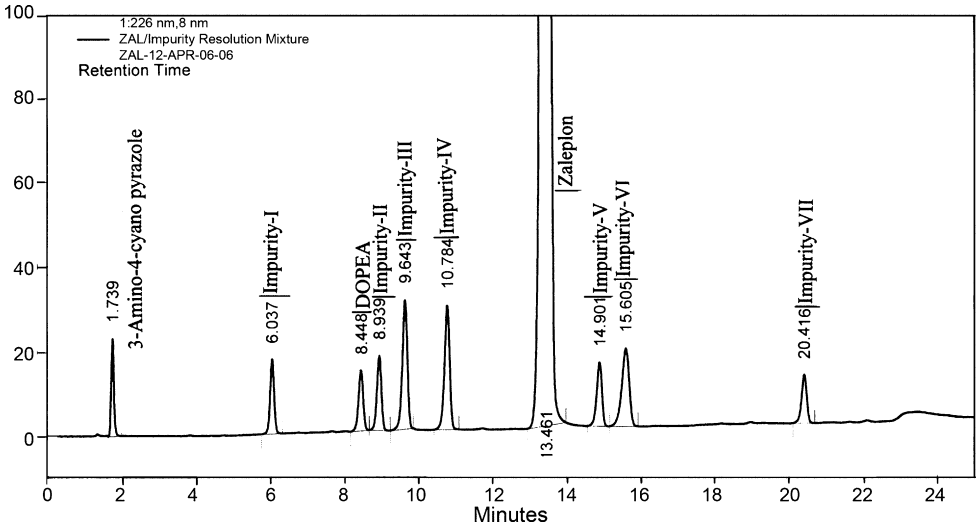


Fig. 2. Typical LC-chromatogram of zaleplon sample spiked with impurities. Column: YMC pack C8, 150 mm × 4.6 mm, mobile phase (A) phosphate buffer (pH 3.0), mobile phase (B) acetinitrile; pump mode: gradient (time (min)/A (v/v): B (v/v); $T_{0.01}$/80:20, $T_{15.0}$/60:40, $T_{25.0}$/45:55, $T_{26.0}$/80:20), flow rate: 1.5 ml/min; wave length at UV, 225 nm.

absent. ^{13}C NMR spectrum showed the absence of signal at 114.2 ppm corresponding to C≡N group. An additional signal was observed at 163.4 ppm corresponding to C=O group. From the above spectral data, it is concluded that the cyano group is replaced by amide group. The molecular formula of this impurity is confirmed as $C_{17}H_{17}N_5O_2$ and the structure was characterized as *N*-[3-(3-carboxamidopyrazolo[1,5-a]pyrimidin-7-yl)phenyl]-*N*-ethylacetamide (zaleplon carboxamide).

3.2.3. Impurity III

ESI mass spectrum of impurity V (RRT-0.70) exhibited a molecular ion peak at *m/z*; 278 [(MH)$^+$] in positive ion mode, indicating the mass of this impurity to be 277 which is 28 amu less than that of zaleplon. This observation suggested the loss of ethyl group from zaleplon. In ^{1}H NMR spectrum of this impurity, the signals at 1.04 ppm and 3.71 ppm assigned to ethyl group in zaleplon were absent. An additional signal at 10.25 ppm which is exchangeable was observed corresponding to one proton as singlet and was assigned to NH group. This was further confirmed in ^{13}C NMR spectrum by the absence of signals at 13.8 ppm and 44.0 ppm which were assigned to ethyl group in zaleplon. Based on the above spectral data, the molecular formula of impurity III was confirmed as $C_{15}H_{11}N_5O$ and the corresponding structure was characterised as *N*-[3-(3-cyanopyrazolo[1,5-a]pyrimidin-7-yl)phenyl]acetamide (Desethyl zaleplon).

3.2.4. Impurity IV

This impurity (RRT ∼0.80) exhibited a molecular ion peak at *m/z* 292 [(MH)$^+$] in positive ion mode. The molecular mass of this impurity is 14 amu less than that of zaleplon. In ^{1}H NMR spectrum, signals corresponding to ethyl group of zaleplon were absent and an additional signal was observed at 3.24 ppm which was integrated for three protons. In ^{13}C NMR spectrum the signals corresponding to ethyl group of zaleplon were absent and an additional signal was observed at 37.4 ppm. From DEPT spectrum it was confirmed as CH_3 signal. From the above spectral data the molecular formula of impurity IV was confirmed as $C_{14}H_9N_5O$ and the corresponding structure was characterised as *N*-[3-(3-cyano pyrazolo [1,5-a]pyrimidin-7-yl)phenyl]-*N*-methylactamide (*N*-methyl zaleplon).

3.2.5. Impurity V

ESI mass spectrum of this impurity showed a molecule ion peak at *m/z* 306 [(MH)$^+$] in positive ion mode which is same as that of zaleplon. This molecular weight suggested that this impurity is an isomer. ^{13}C NMR spectrum is similar to zaleplon with small shift in chemical shift values but appreciable shift is observed in the chemical shift value of carbon at 13 position (Table 3). In ^{1}H NMR spectrum, all signals are similar to those of zaleplon except the appreciable difference in chemical shift values of protons at 6 and 12 position, 8.38 ppm and 8.08 ppm respectively. This spectral pattern confirmed this impurity as a positional isomer and attachment of *N*-ethyl-*N*-acetyl phenyl group on the fused heterocyclic ring system is at 5-position. From the spectral data, the molecular formula of this impurity is confirmed as $C_{17}H_{15}N_5O$ and the structure is characterized as *N*-[3-(3-cyanopyrazolo [1,5-a] pyrimidin-5-yl)phenyl]-*N*-ethyl acetamide (Regioisomer-5).

3.2.6. Impurity VI

This impurity exhibited a molecular ion peak at *m/z* 264 [(MH)$^+$] in positive ion mode, which is less by 42 amu than that of zaleplon. This molecular weight suggests the loss of acetyl group from zaleplon. In ^{1}H NMR spectrum, the singlet at 1.82 ppm is absent which correspond to methyl protons of acetyl group. A triplet is observed at 5.95 ppm which is exchangeable and integrated to one proton. In FT-IR spectrum, amide band at 1650 cm^{-1} is absent and a strong NH band is observed at 3377 cm^{-1}. This observation confirmed the presence of

Table 2
Comparative ^{1}H NMR assignments for zaleplon and its impurities

Position[a]	Zaleplon δ (ppm), multiplicity	Impurity I δ (ppm), multiplicity	Impurity II δ (ppm), multiplicity	Impurity III δ (ppm), multiplicity	Impurity IV δ (ppm), multiplicity	Impurity V δ (ppm), multiplicity	Impurity VI δ (ppm), multiplicity	Position[a]	Impurity VII δ (ppm), multiplicity
1	1.04 (t, 3H)	1.06 (t, 3H)	1.05 (t, 3H)	–	3.24 (s, 3H)	1.05 (t, 3H)	1.18 (t, 3H)	1, 1′	1.02 (t, 6H)
2	3.71 (q, 2H)	3.72 (q, 2H)	3.72 (q, 2H)	–	–	3.73 (q, 2H)	3.08 (q, 2H)	2, 2′	3.70 (m, 4H)
3	–	–	–	–	–	–	–	3, 3′	-
4	1.82 (s, 3H)	1.84 (s, 3H)	1.83 (s, 3H)	2.09 (s, 3H)	1.89 (s, 3H)	1.79 (s, 3H)	–	4, 4′	1.75 and 1.82 (2s, 6H)
5	–	–	–	–	–	–	–	5, 5′	–
6	8.10 (dd, 1H)	8.15 (dd, 1H)	8.15 (d, 1H)	7.84 (d, 1H)	8.08 (dd, 1H)	8.38 (d, 1H)	7.18 (dd, 1H)	6, 6′	8.09-8.13 (m, 2H)
7	7.72 (dd, 1H)	7.71 (dd, 1H)	7.72 (dd, 1H)	7.56 (dd, 1H)	7.72 (dd, 1H)	7.70 (dd, 1H)	7.30 (dd, 1H)	7, 7′	7.69-7.72 (m, 2H)
8	7.60 (dd, 1H)	7.59 (dd, 1H)	7.60 (d, 1H)	7.70 (d, 1H)	7.66 (d, 1H)	7.56 (d, 1H)	6.81 (dd, 1H)	8, 8′	7.62-7.69 (m, 2H)
9	–	–	–	–	–	–	–	9, 9′	–
10	8.04 (s, 1H)	8.04 (s, 1H)	8.07 (s, 1H)	8.33 (s, 1H)	8.08 (s, 1H)	8.21 (s, 1H)	7.22 (s, 1H)	10, 10′	7.72-7.79 (m, 2H)
11	–	–	–	–	–	–	–	11	–
12	7.67 (d, 1H)	7.44 (d, 1H)	7.56 (d, 1H)	7.52 (d, 1H)	7.70 (d, 1H)	8.08 (d, 1H)	7.49 (d, 1H)	12	–
13	8.93 (d, 1H)	8.80 (d, 1H)	8.88 (d, 1H)	8.90 (d, 1H)	8.93 (d, 1H)	9.47 (d, 1H)	8.86 (d, 1H)	13	9.73 (s, 1H)
14	–	–	–	–	–	–	–	14	–
15	–	–	–	–	–	–	–	15	–
16	8.88 (s, 1H)	8.79 (s, 1H)	8.62 (s, 1H)	8.87 (s, 1H)	8.88 (s, 1H)	8.84 (s, 1H)	8.84 (s, 1H)	16	8.83 (s, 1H)
17	–	–	–	–	–	–	–	17	–
18	–	7.56 (brs, 2H)	7.57 (s, 2H)	10.25 (s, 1H)	–	–	5.95 (t, 1H)	18	–
19	–	13.39 (brs, 1H)	–	–	–	–	-	19	7.45 (d (J = 15.6 Hz), 1H)
20	–	8.08 (s, 1H)	–	–	–	–	–	20	8.30 (d (J = 15.6 Hz), 1H)

s, singlet; d, doublet; dd, doublet of a doublet; t, triplet; m, multiplet; brs, broad singlet; q, quartet; J, coupling constant.
[a] Refer structures for numbering (Table 1) and refer Fig. 1 for numbering of zaleplon.

Table 3
Comparative ^{13}C (Proton decoupled) and DEPT NMR assignments for zaleplon and its impurities

Position[a]	Zaleplon		Impurity I		Impurity II		Impurity III		Impurity IV		Impurity V		Impurity VI		Position[a]	Impurity VII	
	^{13}C δ (ppm)	DEPT	^{13}C δ (ppm)	DEPT	^{13}C δ (ppm)	DEPT	^{13}C δ (ppm)	DEPT	^{13}C δ (ppm)	DEPT	^{13}C δ (ppm)	DEPT	^{13}C δ (ppm)	DEPT		^{13}C δ (ppm)	DEPT
1	13.8	CH_3	13.6	CH_3	13.7	CH_3	–	–	37.4	CH_3	13.8	CH_3	15.1	CH_3	1, 1′	13.8	CH_3
2	44.0	CH_2	44.1	CH_2	44.0	CH_2	–	–	–	–	43.9	CH_2	38.0	CH_2	2, 2′	43.9	CH_2
3	169.4	–	170.0	–	170.0	–	169.5	–	169.9	–	169.4	–	–	–	3, 3′	170.0	–
4	23.6	CH_3	23.5	CH_3	23.6	CH_3	24.8	CH_3	23.4	CH_3	23.5	CH_3	–	–	4, 4′	23.6	CH_3
5	147.5	–	146.2	–	147.0	–	148.3	–	147.4	–	150.3	–	149.7	–	5, 5′	148.0	–
6	130.9	CH	130.0	CH	130.4	CH	122.8	CH	130.6	CH	128.5	CH	113.2	CH	6, 6′	130.9	CH
7	132.8	CH	133.5	CH	132.1	CH	130.0	CH	131.0	CH	132.3	CH	117.5	CH	7, 7′	134.3	CH
8	131.7	CH	130.8	CH	130.7	CH	125.1	CH	130.7	CH	131.3	CH	116.0	CH	8, 8′	131.7	CH
9	143.5	–	143.3	–	143.5	–	140.3	–	145.2	–	144.2	–	149.4	–	9, 9′	143.5, 144.0	–
10	130.1	CH	129.7	CH	129.8	CH	120.7	CH	129.3	CH	127.7	CH	130.0	CH	10, 10′	128.5, 129.3	CH
11	151.9	–	147.4	–	147.6	–	151.9	–	151.8	–	159.2	–	152.0	–	11	150.9	–
12	111.8	CH	109.9	CH	110.2	CH	111.5	CH	111.7	CH	109.4	CH	111.3	CH	12	118.5	–
13	154.6	CH	152.2	CH	152.8	CH	154.5	CH	154.5	CH	139.1	CH	154.5	CH	13	154.0	CH
14	131.5	–	132.5	–	132.0	–	130.6	–	131.4	–	137.7	–	130.9	–	14	129.5	–
15	82.3	–	95.0	–	106.3	–	82.2	–	82.3	–	81.9	–	82.0	–	15	83.4	–
16	148.2	CH	146.6	CH	146.6	CH	148.1	CH	148.1	CH	149.2	CH	148.0	CH	16	149.0	CH
17	114.2	–	111.4	–	163.4	–	114.3	–	114.2	–	114.4	–	114.4	–	17	113.9	–
18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	18	188.3	–
19	–	–	–	–	–	–		–	–	–	–	–	–	–	19	125.7	CH
20	–	–	131.6	CH	–	–		–	–	–	–	–	–	–	20	137.0	CH
21	–	–	98.6	–	–	–		–	–	–	–	–	–	–	21	–	–
22	–	–	158.5	–	–	–		–	–	–	–	–	–	–	22	–	–
23	–	–	158.6	–	–	–		–	–	–	–	–	–	–	23	–	–

[a] Refer structures for numbering (Table 1) and refer Fig. 1 for numbering of zaleplon.

Table 4
FT-IR spectral data and Melting range data

S. no.	Compound	IR (KBr) absorption bands (Cm^{-1})	Melting point (°C)
1	Zaleplon	3089, 3033 (w) aryl CH stretch, 2983, 2935, 2877 (w) aliphatic CH stretching, 2233 (s) C≡N stretch, 1652 (s) amide C=O stretching, 1614 (s) aryl C=C stretch and C=N stretch, 801, 698 (m) aryl CH out-of-plane bend	184–186
2	Impurity I	3426 (br and s) NH stretching, 2967 (w) CH_3 stretching, 1634 (s) amide C=O stretching, 1605, 1505, 1486 (s) aryl C=C stretch and C=N stretch, 942, 798 (m) aryl CH out-of-plane bend	–
3	Impurity II	3415 (s) NH stretching, 1652 (s) amide C=O stretching, 1600, 1499 (s) aryl C=C stretch and C=N stretch, 780 (m) aryl CH out-of-plane bend	221–224
4	Impurity III	3321 (s) NH stretching, 3093, 3057 (m) aryl CH stretch, 2237 (s) C≡N stretching, 1688 (s) amide C=O stretching, 1615-1479 (s) aryl C=C stretch and C=N stretch, 785,765 (m) aryl CH out-of-plane bend	256–260
5	Impurity IV	3077, 3028 (w) aryl CH stretching, 2930 (w) CH_3 stretch, 2233 (s) C≡N stretch, 1652 (s) amide C=O stretching, 1614, 1491 (s) aryl C=C stretch and C=N stretch, 804, 701 (m) aryl CH out-of-plane bend	205–206
6	Impurity V	3088, 3064 (w) aryl CH stretching, 2939 (w) CH_3 stretch, 2228 (s) C≡N stretch, 1657 (s) amide C=O stretching, 1626, 1602, 1523 (s) aryl C=C stretch and C=N stretch, 804, 701 (m) aryl CH out-of-plane bend	203–205
7	Impurity VI	3377 (s) NH stretching, 3040 (w) aryl CH stretching, 2973, 2935, 2875 (w) CH_3 and CH_2 stretch, 2235 (s) C≡N stretch, 1610, 1491 (s) aryl C=C stretch and C=N stretch, 1552 (s) NH bending, 804, 693 (s) aryl CH out-of-plane bend	157–160
8	Impurity VII	3062 (s) aryl CH stretching, 2976, 2935, 2876 (w) CH_3 and CH_2 stretch, 2229 (s) C≡N stretch, 1656 (s) amide C=O stretching, 1596, 1580, 1485 (s) aryl C=C stretch and C=N stretch, 806, 706 (s) aryl CH out-of-plane bend.	215–220

w, weak; s, strong; m, medium; br and s, broad and strong.

$NH\text{-}CH_2$ linkage. In ^{13}C NMR spectrum of impurity, the carbon signal at 23.6 ppm which was assigned for acetyl CH_3 in zaleplon was absent. Based on the above spectral data, the molecular formula of this impurity is confirmed as $C_{15}H_{13}N_5$ and the corresponding structure was characterized as *N*-[3-(3-cyanopyrazolo[1,5-a] pyrimidin-7-yl)phenyl]-*N*-ethylamine (Desacetyl zaleplon).

3.2.7. Impurity VII

The ESI mass spectrum of this impurity exhibited a molecular ion peak at *m/z* 521[$(MH)^+$] in positive ion mode. The molecular weight of this impurity is 215 amu more than that of zaleplon. This molecular mass was indicating that this impurity is an adduct of zaleplon and an intermediate, *N*-[3-[3-(dimethylamino)-1-oxo-2-propenyl] phenyl]-*N*-ethylacetamide (DOPEA) with loss of *N*,*N*-dimethylamine. In ^1H NMR spectrum, the signals observed at 1.02 ppm, 1.75 ppm and 3.70 ppm integrated to 6H, 6H and 4H respectively. It was hence confirmed that an additional ethyl group and acetyl group were present in this impurity. An interesting observation to confirm the structure is the presence of two doublets at 7.45 ppm ($J = 15.6$ Hz) and 8.30 ppm ($J = 15.6$ Hz) which were integrated for one proton each. Aryl CH protons were integrated for eight protons indicated the presence of two aryl groups. Doublet at 7.67 ppm in zaleplon disappeared and doublet at 8.93 ppm became singlet and shifted to 9.73 ppm. This spectral pattern confirmed that the attachment was at 6 position of zaleplon molecule. From the above spectral data the molecular formula of this impurity was confirmed as $C_{30}H_{28}N_6O_3$ and the structure was characterized as *N*-[3-(3-cyano-6-[(E)-3-((*N*-ethyl-*N*-acetyl)amino)phenyl-3-oxoprop-1-enyl] pyrazolo[1,5-a]pyrimidin-7-yl) phenyl]-*N*-ethylacetamide (6-propenyloxo zaleplon).

The ^1H and ^{13}C NMR chemical shift values of zaleplon and all impurities are presented in Table 2 and Table 3. The melting range and FT-IR spectral data are given in Table 4.

3.3. Formation of impurities

3.3.1. Impurity I

Starting material, 3-amino-4-cyanopyrazole may have contaminated with dimer impurity, which condenses with DOPEA to yield this impurity.

3.3.2. Impurity II

This impurity originates from the hydrolysis of cyano group during the preparation of zaleplon.

3.3.3. Impurity III

This impurity arises from the condensation of *N*-[3-[3-(dimethylamino)-1-oxo-2-propenyl phenyl acetamide (DOPA) with 3-amino-4-cyanopyrazole. DOPA is present as an impurity in DOPEA intermediate due to incomplete *N*-ethylation reaction.

3.3.4. Impurity IV

This impurity arises due to the presence of methyl halides in ethyl bromide used in zaleplon.

3.3.5. Impurity V

This impurity is a regioisomer of zaleplon. This differs from zaleplon in the position of *N*-ethyl-*N*-acetyl phenyl group on the fused heterocyclic ring system. During formation of zaleplon, the 5-regioisomer originates from the Michael addition of the 2-nitrogen atom of pyrazole onto DOPEA with the elimination of dimethylamine and subsequent cyclization of 3-amino group with the keto group.

3.3.6. Impurity VI

Preparation of zaleplon is done in aqueous acidic medium. This impirity arises due to the acid mediated hydrolysis of acetyl group present in zaleplon.

3.3.7. Impurity VII

During the preparation of zaleplon, it further undergoes electrophilic substitution at 6-position with DOPEA which results in this impurity.

4. Conclusion

The process related impurities in zaleplon bulk drug were identified, isolated and characterized by HPLC (analytical and preparative), LC-MS, FT-IR, and NMR (^{1}H, ^{13}C and DEPT) techniques.

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