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Identification, isolation and characterization of unknown impurity in Ondansetron Drug product

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ABSTRACT

A new degradant of ondansetron was detected at a level of 0.5 % w/w during the reversed phase HPLC analysis in ondansetron 4mg and 8mg stability storage samples. This impurity was identified by LC-MS and was characterized by NMR (^{1}H NMR, ^{13}C NMR and 2D NMR), LC/MS/MS and FTIR spectral studies. This impurity was prepared by isolation from the impurity enriched samples of ondansetron monohydrochloride dihydrate by using reversed phase preparative HPLC. Based on the spectral data, this impurity was named as carboxy (2-methyl-1-((9-methyl-4-oxo-2,3,4,9-tetrahydro-1H-carbazol- 3-yl)methyl)-1H-imidazol-3-ium-3-yl)methanide chloride. The identification, formation and structure elucidation of this impurity has been discussed in details.

Keywords: *Ondansetron, Impurities, Drug-excipient interaction, Characterization, Excipient impurities.*

1. INTRODUCTION

Ondansetron monohydrochloride dihydrate, chemically known as 1,2,3,9-tetrahydro-9- methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one monohydrochloride dihydrate, is a serotonin 5-HT3 receptor antagonist belongs to both imidazole and carbazol family of heterocyclic compound. It is widely used to prevent nausea and vomiting caused by cancer chemotherapy, radiation therapy or surgery [1,2]. It has little effect on vomiting caused by motion sickness. It can be administrated by mouth or by intramuscular or intravenous injections [3,4,5]. The chemical structure of ondansetron monohydrochloride dihydrate is shown in Figure 1.

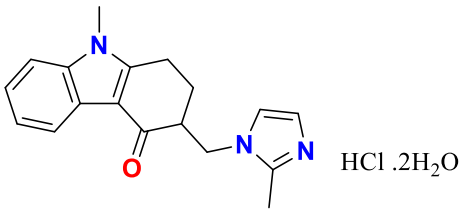

Fig. 1: chemical Structure of Ondansetron monohydrochloride dihydrate

Excipients play an important role in formulating drug products. The formation of the impurities in the drug product is not limited to drug related impurities but there are several possibilities of the drug-excipient interaction as well as excipient impurities reaction with active pharmaceutical ingredients leading to compromised quality or performance of the medication. The level of relative impurities in excipient may vary between lots and suppliers [6,7]. Screening of excipients for these impurities and through understanding of their interaction with active ingredient during early stage of formulation development ensure robust drug product. USA scientist Yongmei Wu et al. has categorized these impurities in six major classes, named reducing sugars, aldehydes, peroxides, metals, nitrate/nitrite, and organic acids. Interactions of these impurities with drug candidate discussed in

details [8]. Ondansetron is a US pharmacopeia listed drug product where five impurities are reported where as in EP monograph eight impurities are known [9,10]. The impurity identified in present work is different from the reported impurities.

In this study, ondansetron 4mg and 8mg tablets were subjected to stability studies as per ICH [12]. During the course of this research work, we analyzed ondansetron stability storage samples; one unknown impurity was observed along with the known impurities at the relative retention time of around 0.32 with respect to the ondansetron peak. The present research work explains the identification, isolation, characterization and formation of this degradation product.

2. EXPERIMENTAL

2.1. Chemicals, Reagents and Samples

Ondansetron monohydrochloride dihydrate and other known impurities were in-house synthesized at Dr. Reddy's Laboratories (Hyderabad, India). Analytical reagents monobasic potassium phosphate, ammonium acetate, acetonitrile, methanol and IR spectroscopy grade potassium bromide were purchased from SD Fine Chemicals Limited (Mumbai, India). Deuterated dimethyl sulfoxide (DMSO-d6) purchased from Aldrich Chemicals Co. (USA). Deionized Water was purified by using Millipore Milli-Q plus purification system.

2.2. High-performance liquid chromatography

The HPLC analysis of ondansetron stability samples were performed with the use of a Waters Alliance 2690 high pressure liquid chromatography system equipped with 2998 photodiode array detector and Waters spherisorb S5CN (250 mm long X 4.6 mm, dp=5 µm) stainless steel column [make: Waters Corporation, Miliford, MA, USA]. 0.02M Phosphate buffer was prepared by dissolving 2.721 g of monobasic potassium phosphate in 1000 ml water, adjusted to pH 5.4±0.05 with 1M solution of sodium hydroxide. Mobile phase and diluent was a mixture of phosphate buffer and acetonitrile in ration of 79: 21 (% v/v). The pump was in isocratic mode and flow rate was kept as 1.5 ml/min, injection volume was 10 µl and the column temperature was maintained at 30°C, chromatographic data acquisition time was 45 min and UV detection was carried out at 216 nm.

2.3. LC/MS/MS Analysis

The LC/MS/MS analysis was carried out using AB SCIEX Triple TOF 4600, Time of Flight mass spectrometer coupled with Agilent 1290 series RRLC system. Analyst ® TF 1.6 software was used for data acquisition and data processing. Ion spray voltage for ion source in ESI mode was maintained at 5500V and temperature was set at 400°C. The auxiliary gas and curtain gas used was high purity nitrogen gas. Zero air was used as nebulizer gas. The resolution of Time of Flight mass spectrometer for ALILTLVS, a synthetic peptide [m/z 829.5398] was more than 25000. The High resolution mass spectra [HRMS] were acquired in the mass range m/z 50-800 in accumulation time of 1000 ms. The analysis was carried out on Waters spherisorb S5CN 250 X 4.6 mm 5 µm stainless column [make: Waters Corporation, Milford, MA, USA] maintained at 25°C. 0.01M ammonium acetate buffer prepared by dissolving 0.77 g of ammonium acetate in 1000 ml water, adjusted to pH 5.4 with acetic acid. Mobile phase was a mixture of ammonium acetate buffer and acetonitrile in ration of 50: 50 (v/v). Diluent was prepared by mixing 500 ml acetonitrile with 500 ml water. The pump was in isocratic mode and flow rate was kept as 1.5 ml/min, injection volume was 20 µl, chromatographic data acquisition time was 30 min and UV detection was carried out at 216 nm.

2.4. Preparative Liquid chromatography

Agilent 1200 series preparative liquid chromatograph equipped with G1315D PDA detector, Rheodyne 2260A series injector with 1.8 ml loop and G3146B Fraction collector [Agilent technologies, Santa Clara, CA, 95051 USA] using YMC Actus Triart Prep C18, 250 mm long, 20 mm i.d., column packed with 10µm particle was employed for isolation of the impurity. Water was used as Mobile phase A and acetonitrile as Mobile phase B. Diluent was prepared by mixing 90 ml methanol with 10 ml water. The flow rate was 15 ml/min, injection volume was 900 µl, chromatographic data acquisition time was 20 min and UV detection was carried out at 216 nm. The HPLC gradient program was set as Time (min)/%B (v/v) : 0.01/20, 5/20, 10/60, 15/60, 16/20, 20/20.

2.5. Nuclear Magnetic Resonance (NMR)

The comparative ^{1}H and ^{13}C NMR spectra for isolated impurity and ondansetron were recorded on a Bruker, AVANCE 400 MHz NMR spectrometer equipped with a 5mm BBO probe and a z-gradient shim system. Deuterated dimethyl sulfoxide (DMSO-d6) with tetramethylsilane (TMS) as an internal standard used as solvent for NMR analysis. ^{1}H and ^{13}C chemical shift values were reported on the δ scale in parts per million (ppm), relative to tetramethylsilane (δ 0.00) and dimethyl sulfoxide (δ 39.50) as internal standards respectively. The H-H bond correlations confirmed by homonuclear shift correlation (gDQCOSY) experiment.

The protonated carbon positions confirmed by hetronuclear single bond (1H-13C, gHSQC) and hetronuclear multiple bond (gHMBC) correlation study. The protonated and non-protonated carbons confirmed by DEPT experiments.

2.6. Fourier Transform Infrared Spectroscopy (FTIR)

The FT-IR spectrum was recorded using Perkin-Elmer spectrum one FT-IR spectrometer (Beaconsfield, UK). Triturated about 4 mg of the sample in about 400 mg of potassium bromide (previously dried at 105°C). A small portion of this sample was made as a pellet with the help of quick press KBr pellet kit (International Crystal Laboratories, Grafield, NJ07026). Recorded IR spectrum between 450 and 4000 cm^{-1} by doing the blank correction using KBr pallet.

2.7. Stability Studies

Ondansetron 4mg and 8mg tablets were subjected to stability studies under formal temperature and humidity conditions, i.e. 40°C(±2°) / 75% RH(±5%) and 25°C(±2°) / 60% RH(±5%). Samples were withdrawn periodically from their respective environmental conditions and impurity profile study was conducted by HPLC, using the procedure mentioned in the HPLC section.

2.8. Excipient Compatibility studies

Ondansetron monohydrochloride dihydrate mixed with sodium starch glycolate in the ratio of 1: 10 % w/w and subjected to wet heat thermal exposure at 60°C for 7 Days. The sample solution was prepared and injected into the HPLC using the procedure mentioned in the HPLC section.

3. RESULTS AND DISCUSSION

3.1. Detection and Identification of the Impurity

The samples described under the stability studies and excipient compatibility studies were diluted to the necessary concentration and was injected into the HPLC using the analytical conditions. An unknown impurity was found along with known impurities [9] [10] at a relative retention time of around 0.32 (RT- 5.339 min) with respect to the ondansetron peak and the same impurity was also observed in the sodium starch glycolate excipient compatibility study with the ondansetron monohydrochloride dihydrate. The level of this impurity was 0.15% w/w to 0.67% w/w at different time intervals. Chromatographic UV spectrum of the unidentified impurity found comparable with ondansetron. However, this impurity was not detected during forced degradation studies of ondansetron drug substance.

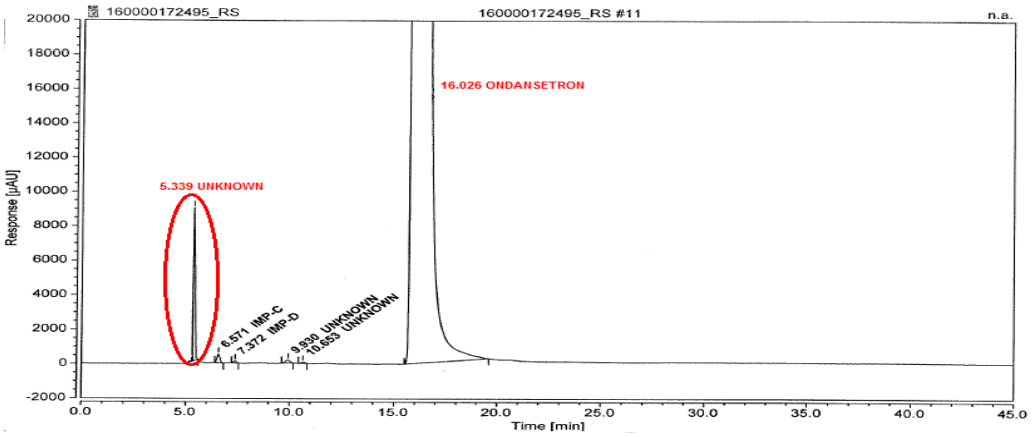

Fig. 2: HPLC chromatogram of ondansetron 4 mg stability storage sample

The unknown impurity at the retention time of around 5.339 min shows the m/z value of 352.1647 when subjected to LC/MS analysis. This impurity was further isolated by preparative HPLC. The preparation, isolation and structure elucidation of the impurity is discussed in the following sections.

3.2. Synthesis and Isolation of the Impurity by preparative HPLC

The unknown impurity was enriched by refluxing 10.0 g of ondansetron monohydrochloride dihydrate with 4 g of AgNO3 and 6 g of chloroacetic in 250 ml of ethyl acetate for about 48hr.This reaction mass was rotary evaporated to dryness and analyzed as per procedure mentioned in the analytical HPLC section. The content of the required impurity found around 10 % w/w.

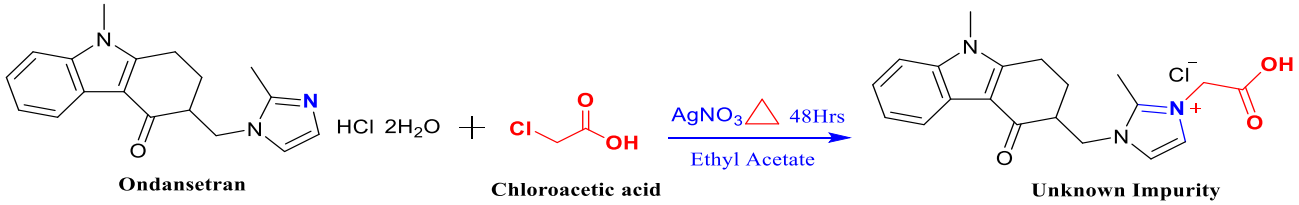

Fig. 3: Synthetic scheme for unknown impurity

About 1500 mg ondansetron monohydrochloride dihydrate reaction mass was dissolved in 25 mL of a diluent (water: Acetonitrile in ratio of 50:50 v/v) and loaded onto the preparative column using conditions mentioned in the "Preparative Liquid Chromatography" section. Fractions of the impurity ≥99.0% (time window at around 4–6 min) were pooled mutually and concentrated on the rotavapor to remove the organic solvent. The aqueous phase was expelled by lyophilization using the Virtis advantage 2XL freeze dryer. The impurity was achieved with chromatographic purity of 98.11% determined by the HPLC method.

3.3. Structural Elucidation of the Impurity

The electrospray ionization mass spectrum of the unknown impurity shows the m/z value 352.1647 amu in positive ion mode by LC-MS analysis, whereas the mass of ondansetron is 293.1914 amu. The difference between these two mass values is 59 amu, found matching with the mass of propionic acid moiety. The chemical atom positions of ondansetron monohydrochloride dihydrate and the proposed structure of the impurity are represented in Fig. 4. The chemical shift (δ) values [presented in Table 1] obtained from ^{1}H NMR and ^{13}C NMR spectrum of ondansetron monohydrochloride dihydrate and the impurity infers the following observations.

Fig. 4: Structure of Ondansetron monohydrochloride dihydrate and unknown impurity

Structure of the isolated impurity confirmed by comparing ^{1}H and ^{13}C NMR signals with those of ondansetron.^{1}H NMR spectrum of the impurity depicts sharp singlet signal at 4.39 ppm corresponds to CH_2 group of propionic acid moiety which is not present in the ^{1}H NMR spectrum of ondansetron monohydrochloride dihydrate.

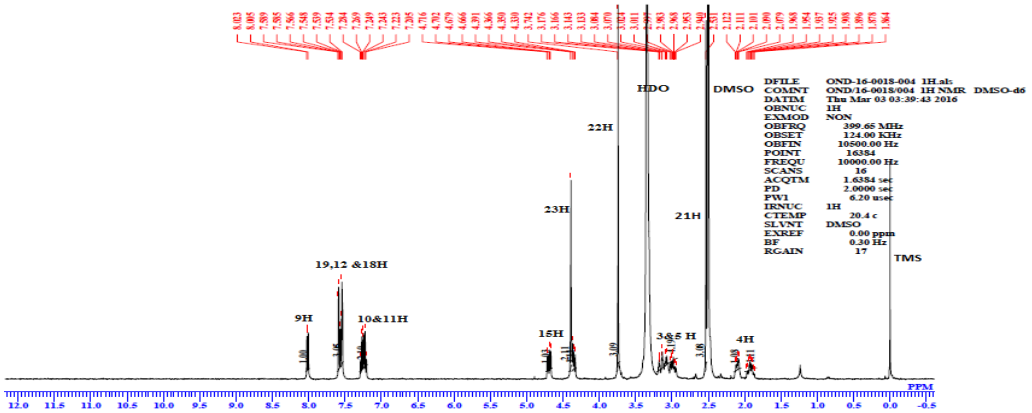

Fig. 5: ^{1}H NMR Spectrum of the impurity

^{13}C NMR spectrum of the impurity demonstrated additional signals at δ 52.3 and δ 166.3 ppm which are absent in ondansetron monohydrochloride dihydrate. Additionally, slightly down filed chemical shift value was observed for ^{13}C NMR signals corresponds to imidazole moiety (position 18) revealed information about the site of attachment of propionic acid moiety.

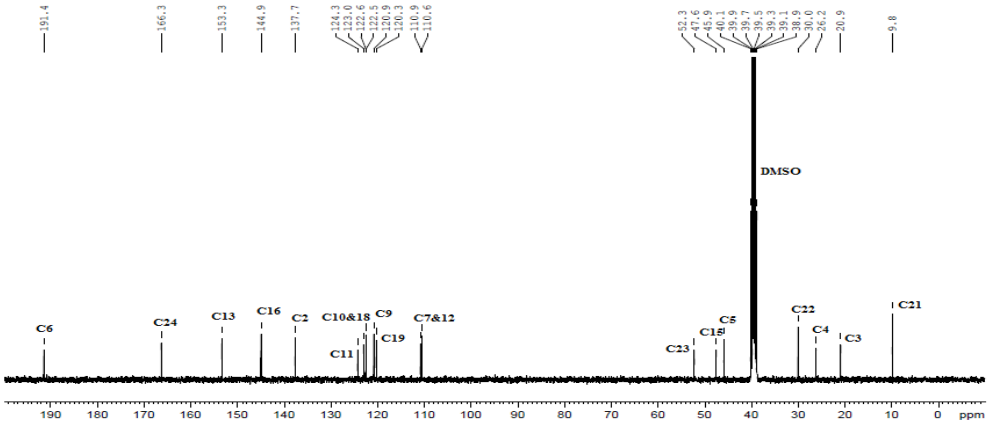

Fig. 6: ^{13}C NMR Spectrum of the impurity

Signal appeared at 52.3 ppm in the ^{13}C NMR spectrum of the impurity depict below the plane signal in DEPT-135 study, whereas signal at δ 166.3 ppm confirmed as quaternary carbon atom. Below the plane signal at δ 52.3 ppm corresponds to C-23 methylene CH_2 shows co-relation with sharp singlet signal at δ 4.39 in gHSQC study, this phenomenon explain presence of propionic acid moiety.

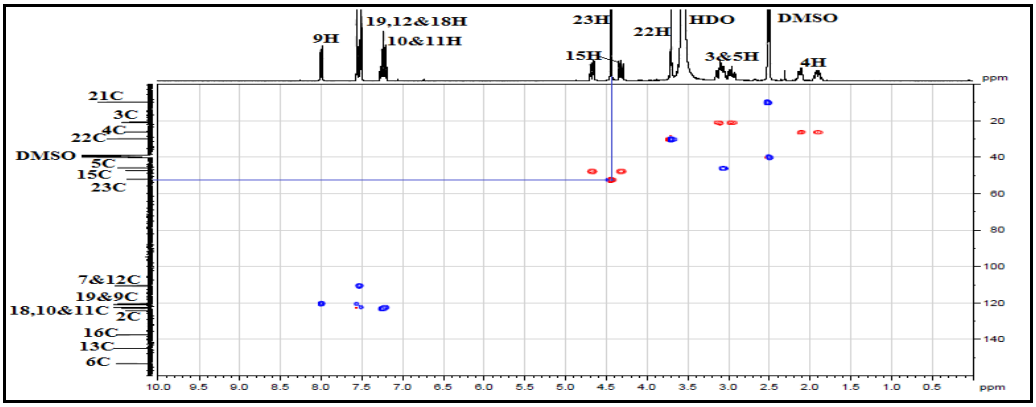

Fig. 7: gHSQC NMR spectrum of the impurity

Further confirmation for the structure of the impurity was obtained from long-range proton carbon coupling experiment (gHMBC). The cross peak of H-23 with those of quaternary carbon C-24, C-16 and C-18 positions (Fig.9) confirm the point of attachment of propionic acid moiety. These interactions are shown in Table 1.

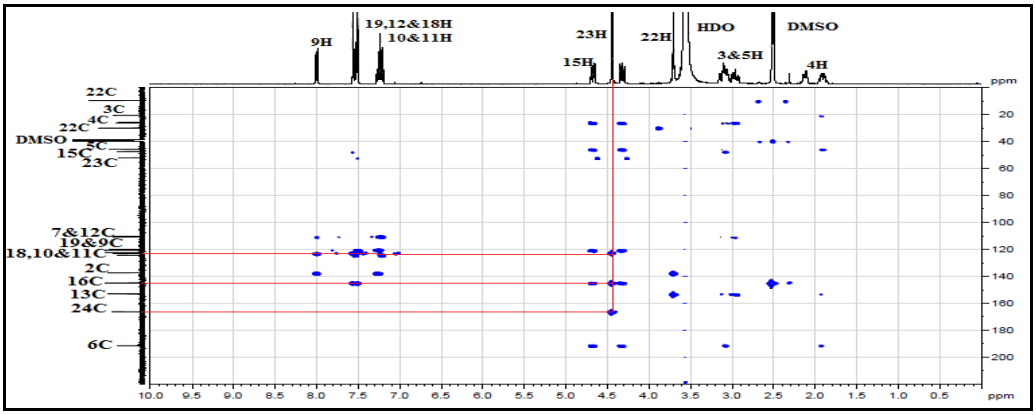

Fig. 8: gHMBC NMR spectrum of the impurity

Table-1: 1D and 2D NMR assignments for Ondansetron and the impurity

Ondansetron			**Impurity**			
Position	1H δ (ppm), multiplicity, J_{1-2}	13C δ (ppm)	1H δ (ppm), multiplicity, J_{1-2}	13C δ (ppm)	gHSQC	gHMBC
1	----	----	----	----	----	----
2	----	153.0	----	153.3	----	----
3	2.95-3.18 (m,2H,5.2)	20.9	2.94-3.18 (m,2H,5.2)	20.9	3H, 2.940-3.176	C-2,4,5,7
4	1.92-2.17 (m,1H,4.8)	26.3	1.86-2.12 (m,1H,4.8)	26.2	4H, 1.864-2.122	C-3,5,2,6,15
5	3.02-3.18 (m,1H)	45.4	3.01-3.18 (m, 1H)	45.9	5H, 3.011-3.176	C-4,6,15,3
6	----	191.4	----	191.4	----	----
7	----	110.6	----	110.6	----	----
8	----	124.1	----	124.3	----	----
9	7.99-8.01 (d,1H,7.6)	120.1	8.01-8.02 (d,1H,7.6)	120.9	9H, 8.005-8.023	C-8,10,7,11,13
10	7.20-7.28 (t,1H,6.8)	122.3	7.21-7.28 (t,1H,6.8)	122.6	10H, 7.205-7.284	C-9,11,8,12
11	7.20-7.28 (t,1H,6.8)	122.7	7.21-7.28 (t,1H,6.8)	123	11H, 7.205-7.284	C-10,12,9,13
12	7.55-7.57 (d,1H,6.8)	110.4	7.55-7.57 (t,1H,6.8)	110.6	12H, 548-7.566	----
13	----	137.4	----	137.7	----	----
14	----	----	----	----	----	----
15	4.33-4.72 (m,2H,7.2)	46.9	4.33-4.72 (m, 2H)	47.6	15H, 4.330-4.716	C-5,6,16,19
16	----	144.5	----	144.9	----	----
17	----	----	----	----	----	----
18	7.59-7.59 (d,1H,2.4)	117.7	7.53-7.54 (d, 1H)	120.3	18H, 7.534-7.539	C-19,16,23
19	7.69-7.69 (d,1H,2.0)	122.5	7.59-7.59 (d, 1H)	122.5	19H, 7.585-7.589	C-18,20,19
20	----	----	----	----	----	----
21	2.66 (s, 3H)	10.4	2.53 (s, 3H)	9.8	21H, 9.8	C-16
22	3.74 (s, 3H)	29.8	3.74 (s, 3H)	30	22H, 3.74	C-2,13
23	----	----	4.39 (s, 2H)	52.3	23H, 4.391	C-24,16,18
24	----	----	----	166.3	----	----
s…singlet; d…doublet; dd…doublet of doublet; m…multiplet; brs…broad singlet; q…quartet; t…triplet; Refer to structures (Fig. 4) for numbering purposes						

The high-resolution mass spectrometry for the mass m/z 352.1647 recommended the elemental composition $C_{20}H_{22}N_3O_3$ and major fragments at m/z 240.1, 212.1, 184.9 and170.1, complies with the proposed structure of the impurity. The fragmentation is shown in Fig. 9.

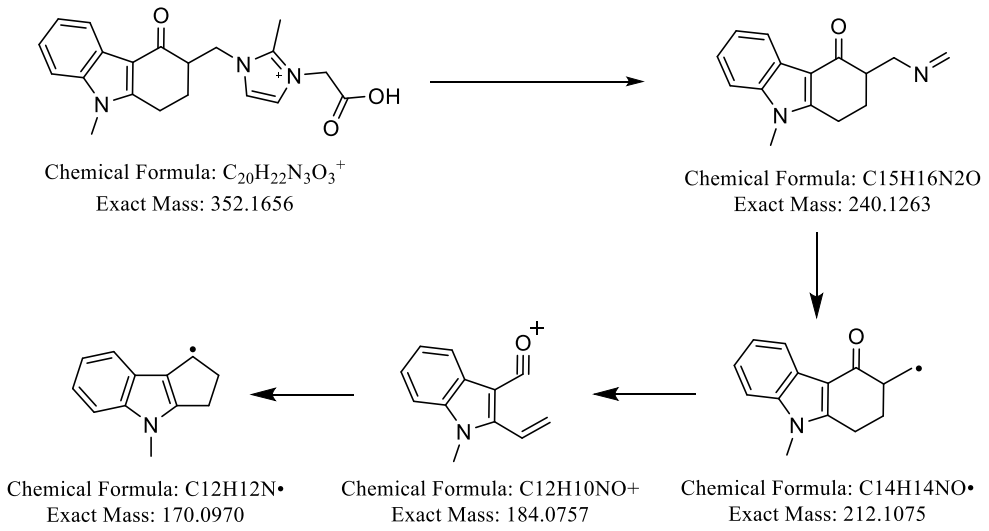

Fig. 9: The proposed fragmentation pathway of the unknown impurity

In addition to that, this impurity was further confirmed by FTIR spectral data. Comparative FTIR data of the impurity with ondansetron are summarized in Table 5.

Table 2: FT-IR spectral data for Ondansetron and unknown Impurity

Ondansetron		**Impurity**	
3500(b)	Free OH Stretching	3478(b)	HO Stretching
3139(s)	aromatic C-H stretching	3132(s)	aromatic C-H stretching
2926(s)	aliphatic C-H stretching	2931(s)	aliphatic C-H stretching
1740(s)	C=O Stretching	1738(s) & 1716(s)	C=O Stretching
1623(s)	-C=N stretching	1623(s)	C=N stretching
1531(s)	Aromatic C=C stretching	1531(s)	Aromatic C=C stretching
1478(s)	aliphatic C-H bending	1482(s)	aliphatic C-H bending
1279(s)	C-N stretching	1273(s)	C-N stretching
854(s)	aromatic C-H bending	854(s)	aromatic C-H bending
757(s)	di-substituted benzene	757(s)	di-substituted benzene

4. FORMATION OF THE IMPURITY

This degradation product of ondansetron originates due to interaction of ondansetron with chloroacetic acid impurity present in the sodium starch glycolate. The lone pair of electron on the nitrogen atom in an imidazole ring of ondansetron molecule is attracted towards the + carbon of the chloroacetic acid (halogenoalkane) and forms a bond with it. In this process expelling chlorine as a counter ion.

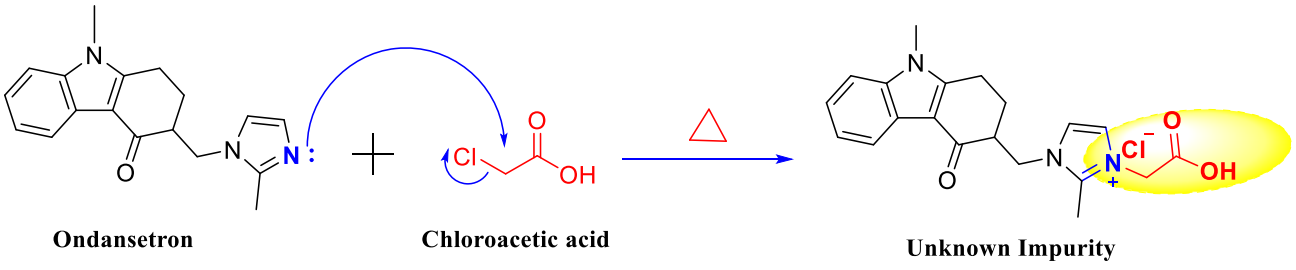

Fig. 10: Mechanism for impurity formation

5. CONCLUSION

Ondansetron 4mg and 8mg tablets were subjected to stress stability studies and the degradation product evaluated. One unknown impurity at RRT 0.32 found along with other known impurities. This Impurity was synthesized by isolation form ondansetron dihydrochloride monohydrate treated with chloroacetic acid. Chromatographic UV spectrum and fragmentation pattern of the unknown impurity found comparable with ondansetron, except the parent ion peak in the mass spectroscopy. Sharp singlet signal at 4.39 ppm in ^{1}H NMR spectroscopy and ^{13}C NMR signals at δ 52.3 and δ 166.3 ppm suggest attachment of propionic acid moiety to the ondansetron molecule. Position of attachment confirmed by hetronuclear single bond (1H-13C, gHSQC) and hetronuclear multiple bond correlation (gHMBC) experiments. Presence of chloride as counter ion confirmed by silver nitrate identification test. Based on available spectral data (NMR, Mass and FTIR) probable structure of the impurity identified as carboxy(2-methyl-1-((9-methyl-4-oxo-2,3,4,9-tetrahydro-1H-carbazol-3-yl)methyl)-1H-imidazol-3-ium-3-yl)methanide chloride.

6. ACKNOWLEDGEMENT

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BIOGRAPHY

Hemant Madhusudan Gandhi

Principle Scientist

I am working as principle scientist at Dr Reddys laboratory. I have 15 years' experience in advance instrumentation laboratory. Much of the research work has done in plant investigations, identification synthesis and structure elucidation of novel unknown impurities, solid state chemistry, characterization of NDDS formulations and Extractable leachable studies for container closer systems.

Prof. G. Nageswara Rao

Vice Chancellor

Prof. G. Nageswara Rao is Vice Chancellor of Andhra university science 17th July 2016. He has done extensive work on separation of metal complexes, chelates and ligands having more than 300 publications in various national and international journal. He was awarded "Biotechnology National Associate" by Department of Biotechnology, Govt. of India. He worked as Post-Doctoral Research Fellow at University of Durban, South Africa during 1997-98. Recognizing the research talent and academic pursuits in the form of good publications, Andhra University conferred Best Researcher Award in 2000.

