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IDENTIFICATION, ISOLATION AND CHARACTERIZATION OF POTENTIAL DEGRADATION PRODUCT IN LANSOPRAZOLE DRUG SUBSTANCE

K.Ramulu[1], B.M. Rao[2*] and N.Someswara Rao[2]

[1]Analytical Research, Custom Pharmaceutical Services, Dr. Reddy's Laboratories Ltd. Bollaram Road, Miyapur, Hyderabad, 500049, India
[2]Andhra University, Visakhapatnam-530 003, India
[*]E-Mail: drbmrao@gmail.com

ABSTRACT

In the stress degradation studies of Lansoprazole, one major unknown acid degradation impurity was identified by LC-MS. This impurity was isolated using preparative high performance liquid chromatography. Based on the spectral data (^{1}H NMR, ^{13}C NMR, DEPT, COSY, HSQC, HMBC, MS, HR-MS and IR), this degradation impurity is characterized as 1-Methyl-10-thioxo-10H-4a,5,9b-triaza-indeno[2,1-a]inden-2-one. The details of stress studies, identification, isolation, characterization, formation and mechanism of this impurity is discussed and presented here.

Keywords: Lansoprazole, Degradation, Potential degradation product, Identification, Isolation, Characterization

INTRODUCTION

Lansoprazole is a proton pump inhibitor and it is belongs to class of substituted benzimidazole. Its IUPAC name is 2-[[[3-methyl-4- (2, 2, 2-trifluoro ethoxy)-2-pyridyl] methyl] sulfinyl] benzimidazole. Lansoprazole is used for treating ulcers of the stomach and duodenum. It is also used for treatment of gastroesophageal reflux disease (GERD) and Zollinger-Ellison Syndrome. Like, other proton-pump inhibitor, Lansoprazole blocks the enzyme in the wall of the stomach that produces acid. Lansoprazole appears to be superior to Ranitidine in treating reflux oesophagitis[1]. In patients who require continuous treatment with nonsteroidal anti-inflammatory drugs, Lansoprazole is superior to Ranitidine for healing of nonsteroidal anti-inflammatory drugs associated gastric ulcers[2].

Several analytical methods have been reported for Lansoprazole impurities and assay determination in the bulk drug, pharmacokinetics and formulations[3-8]. Lansoprazole determined in human plasma by liquid chromatography-tandem mass spectrometry[9-10]. Lansoprazole related impurities determination method is available in United States Pharmacopeia monograph[11]. Madhusudhan Reddy et al. and Srinivas et al. published on characterization of process related impurities of Lansoprazole[12-13]. Selenka et al. published Lansoprazole degradation products identification by LC-MS[14]. To the best of our knowledge there are no reports on the characterization of degradation products of Lansoprazole in literature. The present research work describes the identification, isolation and characterization of major unknown degradation product formed in the acid degradation studies[15].

EXPERIMENTAL

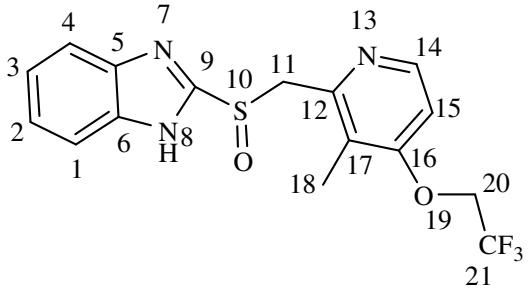
Chemicals and Reagents

The investigated samples of Lansoprazole were received from Process Research Department of Custom Pharmaceutical Services of Dr. Reddy's Laboratories Limited, Hyderabad, India. The chemicals used in the present analysis are triethylamine (Rankem), sodium hydroxide (Rankem), hydrochloric acid (Rankem), hydrogenperoxide (S.D. Fine Chemicals) and HPLC-grade acetonitrile (Rankem). Water used in the analysis and isolation was purified using Milli-Q plus purification system.

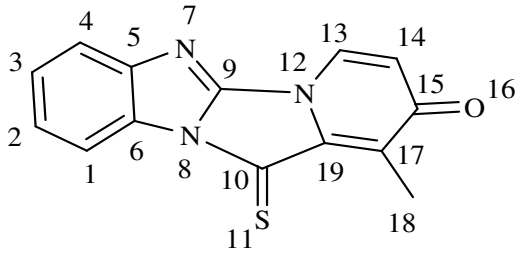
Instrumentation

High-performance liquid chromatography

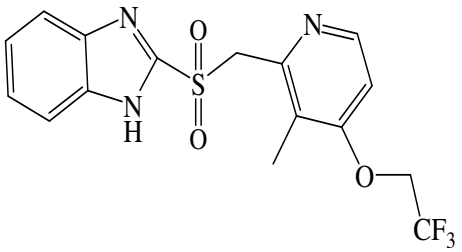
An Agilent 1100 series HPLC with photodiode array detector with chemstation data handling system was used. A reverse phase gradient HPLC method was adopted from the United States pharmacopeia monograph using YMC Pack Pro C18, 150 mm x 4.6 mm, 5 µm (YMC Inc., USA) column for the Lansoprazole impurities determination and stress degradation studies. The used conditions are as follows. Mobile phase A was water. Mobile phase B was acetonitrile, water and triethylamine in the ratio of 160:40:01 (v/v/v) and adjusted to pH=7.0 with phosphoric acid. UV detection was carried out at 285 nm and flow rate was kept at 0.8 mL/min. Column oven temperature was maintained at 25°C.The gradient program: time / % of MP-B was 0/10, 40/80, 50/80, 51/10,60/10.



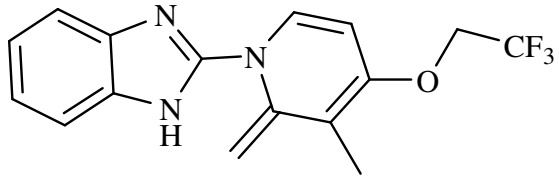
Lansoprazole



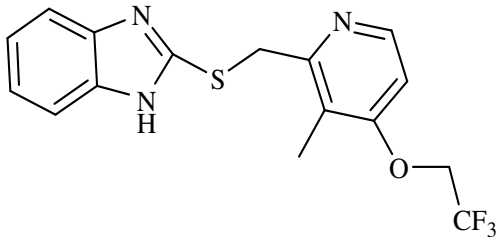
Degradation product



Related compound A (Sulfone impurity)



Impurity B (Des-sulfur impurity)



Impurity C (Sulfide impurity)

Fig.-1: Chemical structures of Lansoprazole and impurities

High-performance liquid chromatography (preparative)

An Agilent 1100 series preparative liquid chromatography equipped with photodiode array detector system was used. Data was processed through chemstation software. Zorbax SB C18 (250mm long×9.4mm i.d.) preparative column packed with 5µm particle size was employed for isolation of the impurity. Mobile phase A was water and acetonitrile in the ratio of 80:20 (v/v) and mobile phase B was acetonitrile. The flow rate was kept at 10 mL/min and the UV detection was carried out at 285 nm. The gradient program employed with T (min) / % mobile phase-B (v/v) as 0/15, 15/50, 17/90, 19/90, 20/15 with post run time of 5 minutes.

LC-MS
LC-MS analysis was carried out on the acid degraded drug substance of Lansoprazole using following conditions. Water as a mobile phase A and acetonitrile and water in the ratio of 160:40 (v/v) as mobile phase B was used. The gradient/elution (T/%B: 0/10, 40/80, 50/80, 51/10, 60/10), flow rate of 0.8 mL/min and detection at 285nm was used. An YMC Pack Pro C18 column (150mm×4.6mm i.d., with a particle size of 5µm) was used. The injected volume was 10µL. Nitrogen as nebulizer gas and as collision assisted dissociation gas was used with Capillary voltage 4000V and collision energy 25V. Source gas temperature at 325°C and flow rate at 12 lit/min was used. The data were processed using Mass hunter software.

High resolution mass
Samples were analyzed on the Micromass LCT Premier mass spectrometer equipped with an ESI Lockspray source for accurate mass values. Leucine enkephalin was used as internal reference compound which was introduced via the Lockspray channel using the Waters reagent manager. The mass range was calibrated with the cluster ions of sodium formate using a fifth order polynomial fit. The data were acquired in W mode using the Mass Lynx software.
The mass spectrometer was equipped with a Waters Acquity system. The conditions were used for the analysis are as follows. Water as a mobile phase A and acetonitrile and water in the ratio of 160:40 (v/v) as mobile phase B was used. The gradient/elution (T/%B: 0/10, 40/80, 50/80, 51/10, 60/10), flow rate of 0.8 mL/min and detection at 285nm was used. An YMC Pack Pro C18 column (150mm×4.6mm i.d., with a particle size of 5µm) was used. Impurity was dissolved in water: acetonitrile (50:50 v/v) diluent at a concentration level of 0.25 mg/mL. The positive electrospray data were obtained by capillary voltage 2400 V, cone voltage 70V, cone gas 50 L/min, source temperature 125°C, desolvation temperature 250°C and desolvation gas flow 450 L/h**.**

NMR spectroscopy
The NMR spectra for Lansoprazole and degradation product were recorded on Varian Mercury plus 400 MHz, Gemini 200 MHz (Gemini-2000) and Unity Inova 500 MHz NMR spectrometers (Varian, Germany) in DMSO-*d*6 and $CDCl_3$ solvents. The ^1H chemical shift values were reported on the δ scale in ppm, relative to TMS (δ=0.00ppm), while ^{13}C chemical shift values were reported relative to DMSO-d_6 (δ=39.50ppm) and $CDCl_3$ (δ=77.0 ppm) as internal standards. Distortionless enhancement by polarization transfer (DEPT) spectral editing revealed the presence of methyl and methyne groups as positive peaks while the methylenes as negative peaks. The data were processed using Linux software.

ESI-MS
The Mass spectra were recorded on Agilent 6410 Triple Quadrupole mass spectrometer. The sample was introduced through a union from liquid chromatograph into mass spectrophotometer in positive ionization mode of Electro spray ionisation. Nitrogen gas was used in nebulizer with Capillary voltage 4000V. Source gas temperature at 325°C and flow rate at 12 lit/min was used. The data were processed using Mass hunter software.

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

Preparation of acid degradation sample
A solution of Lansoprazole (500 mg) in 25mL of 0.1N hydrochloric acid was kept for 24 h.

RESULTS AND DISCUSSION

Analysis of acid degradation sample by analytical HPLC
The acid degradation sample was diluted to required concentration and analyzed with analytical high-performance liquid chromatography. The analytical HPLC chromatograms of Lansoprazole and stressed

sample in acid degradation are shown in Fig.-2 and Fig.-3 respectively. The Lansoprazole eluted about a retention time of 27 min. In the chromatogram of the acid stressed sample, one prominent unknown degradation impurity was found about 32% by area normalization at relative retention time of 1.17 with respect to Lansoprazole peak.

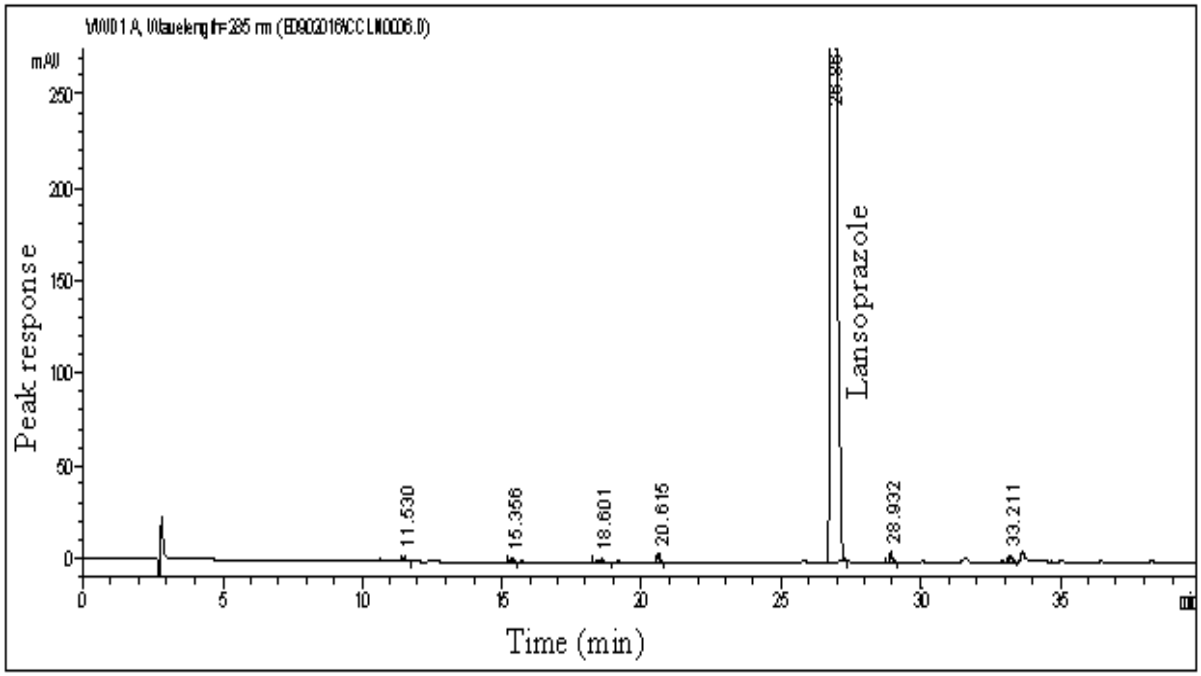


Fig.-2 : HPLC Chromatogram of Lansoprazole

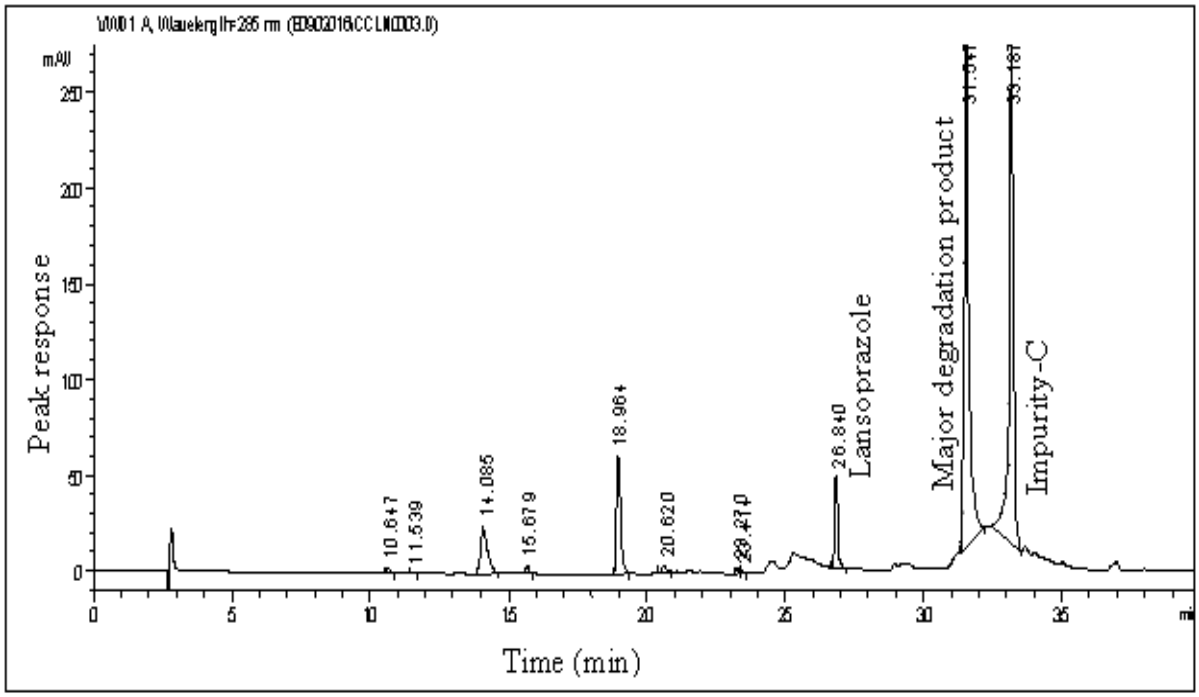


Fig.-3 : HPLC Chromatogram of stressed sample by acid hydrolysis condition

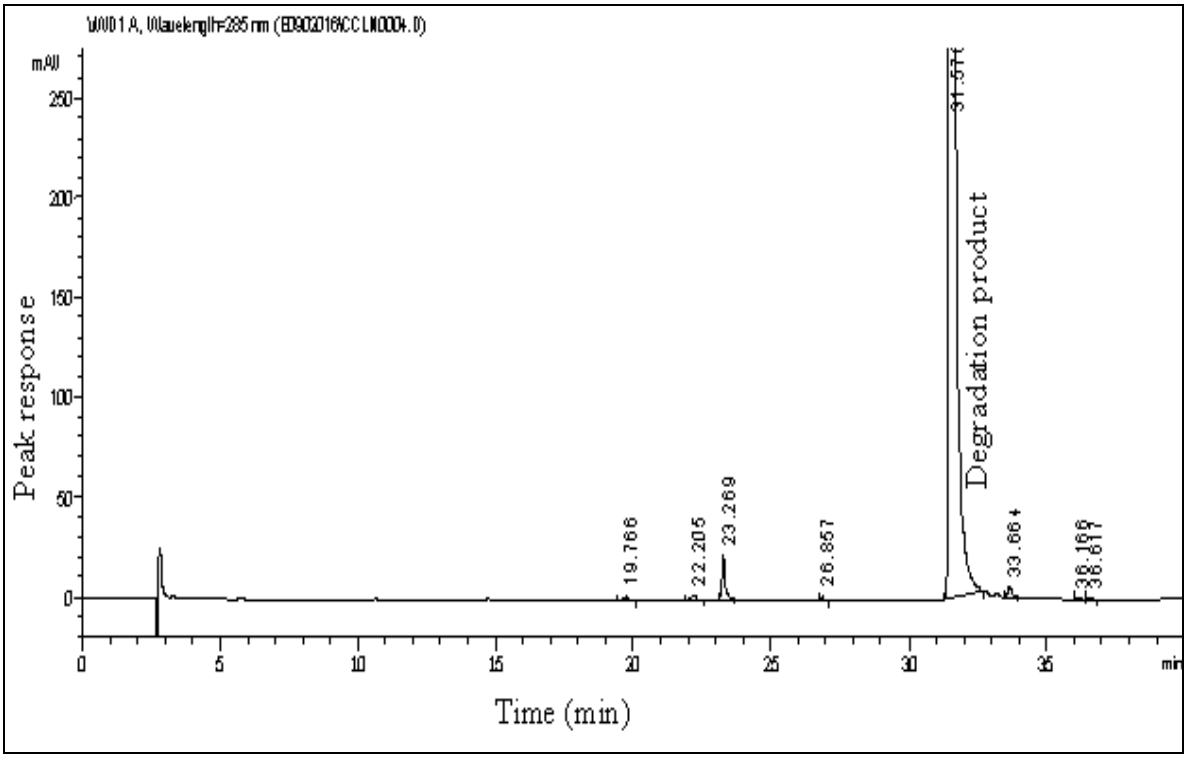


Fig.-4 : HPLC Chromatogram of acid degradation product

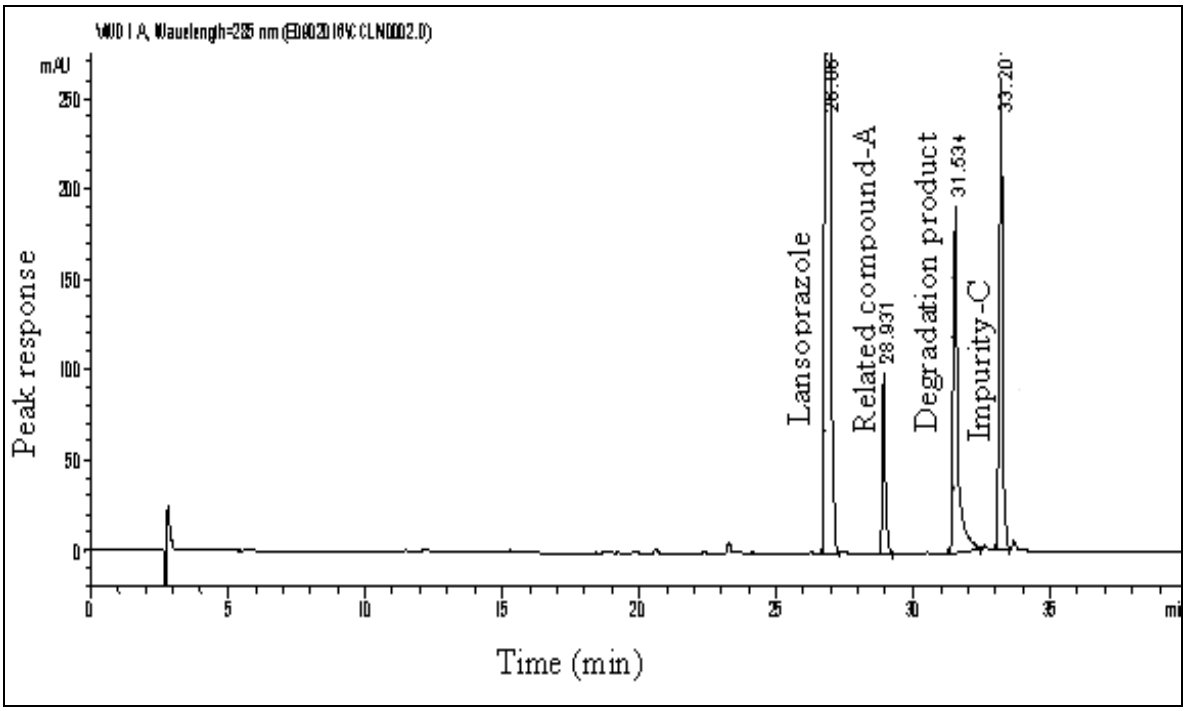


Fig.-5: Lansoprazole spiked with degradation product and known impurities

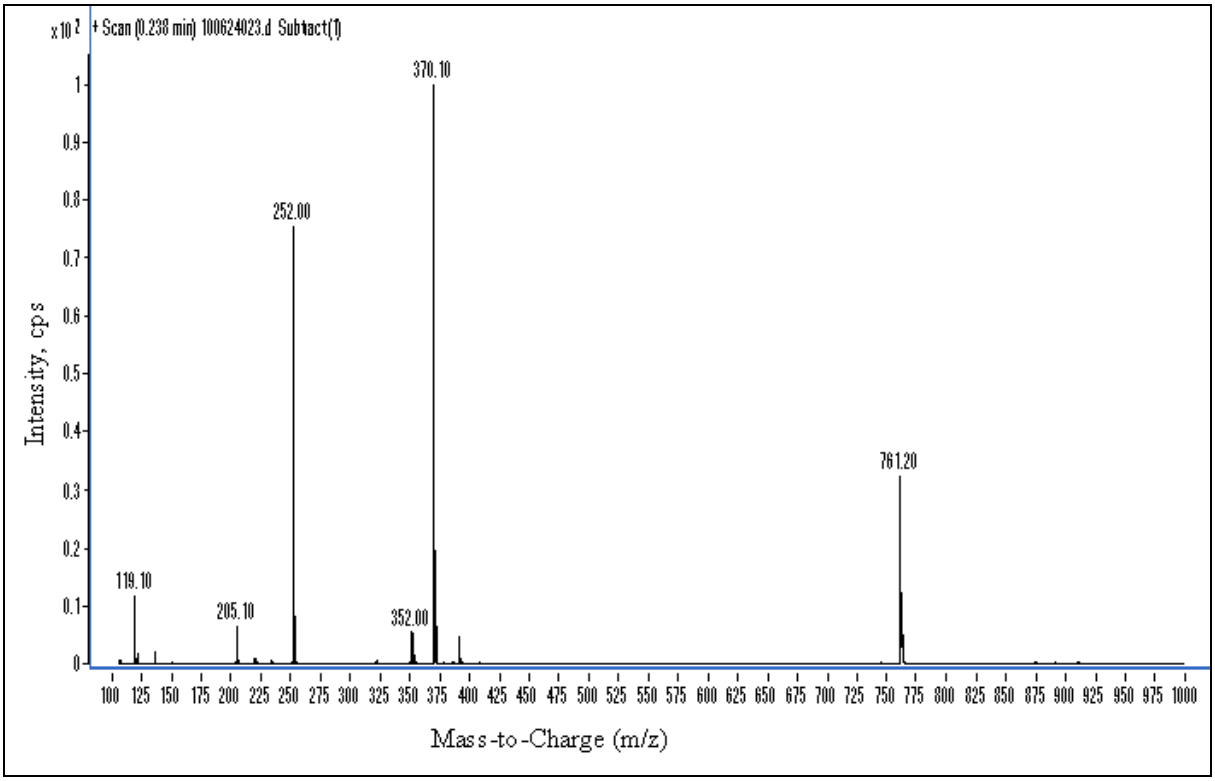


Fig.-6: Mass spectrum of the Lansoprazole

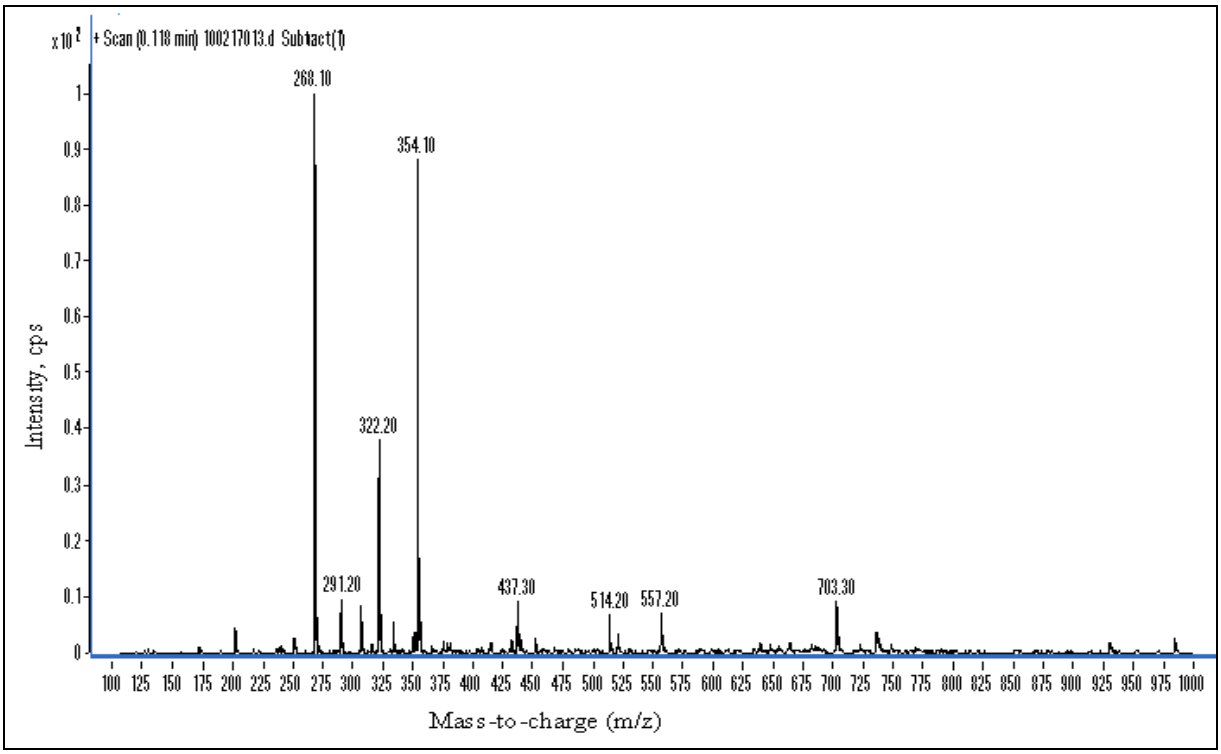


Fig.-7: Mass spectrum of the degradation product

Table -1: NMR assignments of Lansoprazole and Degradation product

Position[1]	Lansoprazole					Degradation product				
	^{1}H	δ (ppm)	^{2}J	^{13}C	DEPT	^{1}H	δ (ppm)	^{2}J	^{13}C	DEPT
1	1H	7.59/s	-	122.65	-CH	1H	8.16/d	7.6	113.28	-CH
2	1H	7.31/s	-	112.41	-CH	1H	7.34/t	7.6	125.54	-CH
3	1H	7.31/s	-	119.64	-CH	1H	7.40/t	7.6	127.11	-CH
4	1H	7.72/s	-	123.81	-CH	1H	7.62/d	7.6	120.52	-CH
5	-	-	-	143.02	-	-	-	-	146.89	-
6	-	-	-	134.72	-	-	-	-	129.23	-
8	1H	13.57/s	-	-	-	-	-	-	-	-
9	-	-	-	154.17	-	-	-	-	149.94	-
10	-	-	-	-	-	-	-	-	177.93	-
11	2H	4.79/dd	13.6	59.99	$-CH_2$	-	-	-	-	-
12	-	-	-	150.92	-	-	-	-	-	-
13	-	-	-	-	-	1H	8.00/d	7.6	128.72	-CH
14	1H	8.29/d	5.5	148.13	-CH	1H	6.48/d	7.6	116.12	-CH
15	1H	7.09/d	5.9	107.04	-CH	-	-	-	180.65	-
16	-	-	-	161.31	-	-	-	-	-	-
17	-	-	-	122.09	-	-	-	-	134.18	-
18	3H	2.18/s	-	10.55	$-CH_3$	3H	2.61/s	-	9.72	$-CH_3$
19	-	-	-	-	-	-	-	-	139.72	-
20	2H	4.91/q	8.8	64.65(q)	$-CH_2$	-	-	-	-	-
21	-	-	-	123.77(q)	-	-	-	-	-	-

1: Refer Fig.-1 for numbering
2: This column gives ^{1}H- ^{1}H coupling constant
3: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (dd) doublet of doublet

Analysis of acid degradation sample by LC-MS

To get structural insight, the LC-MS analysis was carried out on the acid stressed sample. The mass spectrum thus obtained was shown the protonated molecular ion of degradation impurity at m/z 268, whereas the Lansoprazole displayed protonated molecular ion at m/z 370. Thus the degradation impurity has 102 amu less than the molecular ion of Lansoprazole.

As the molecular ion information is not just enough to arrive at the structure, the drug substance was stressed and subjected to purification by preparative HPLC to isolate more quantity of degradation impurity for spectroscopic studies.

Table -2: FT-IR spectral data of Lansoprazole and Degradation product

Compound	IR[a] Characteristic Absorptions (cm^{-1})
Lansoprazole	3236 (N-H stretching), 2984 (aromatic C-H stretching), 2930 (aliphatic C-H stretching), 1580 (aromatic C=C stretching), 1267 (C-F stretching), 1173 (S=O stretching), 1118 (C-O stretching), 1038 (C-N stretching).
Degradation product	2926 (aromatic C-H stretching), 2870 (aliphatic C-H stretching), 1722 (C=O stretching), 1578 (aromatic C=C stretching), 1184 (C=S stretching), 1093 (C-N stretching).

[a] KBr

Isolation of degradation product by preparative HPLC

Lansoprazole (1.0g) was treated with hydrochloric acid (0.1N, 25mL) and kept at room temperature for 24 h. Degraded solution was subjected to preparative HPLC. Fractions collected were analyzed by analytical HPLC. Fractions of >95% purity were pooled together and concentrated on rotavapour to remove the solvent mixture. To confirm the retention time of the isolated impurity the isolated fraction was analyzed by analytical HPLC. The impurity obtained as red color solid and the chromatographic purity is 96.0% by area percentage. Isolated impurity (Fig.-4) was spiked to Lansoprazole along with the known impurities and spiked HPLC chromatogram was shown in (Fig.-5).

Structure elucidation of degradation product

The HR MS data of this degradation product showed exact mass of the protonated molecular ion at m/z 268.0552 (Calcd.268.0545) for $C_{14}H_{10}N_3OS$ which corresponds to the molecular formula $C_{14}H_9N_3OS$.

The ^1H NMR, ^{13}C NMR and DEPT spectral data of degradation product was compared with those of Lansoprazole in Table-1. The numbering scheme for the NMR assignments is shown in Fig-1. In ^1H NMR, presence of -CH_2 (methylene) protons at 20th position in Lansoprazole, are missing in degradation impurity. As well, in ^{13}C NMR, presence of -CH_2 (methylene) group carbon at 20th position in Lansoprazole, is missing in degradation impurity. In ^1H NMR, the presence of -CH_2 protons at 11th position in Lansoprazole are missing in degradation impurity. In ^{13}C NMR, the presence of -CF_3 group at 21st position in Lansoprazole is also not seen in degradation impurity. In ^{13}C NMR of impurity, the formation of carbonyl group at 15th position is observed, where as it is not presented Lansoprazole. It is also observed in ^{13}C NMR spectrum of the degradation impurity is formation of -C=S (thione) group at 10th position, where as it is not observed in Lansoprazole. DEPT NMR spectral data (Table-1) also revealing that -CH_2 (methylene) groups presented in Lansoprazole at 11th and 20th positions are absent in degradation impurity. ^1H-^1H connectivities and ^{13}C-^1H connectivities in the degradation product was confirmed by COSY, HSQC and HMBC NMR spectral data. Thus the degradation impurity structure can be rationalized in terms of formation of cyclic thione ring and the formation of carbonyl group.

The electrospray ionization (ESI) mass spectrum of the degradation product (Fig-7) exhibited a molecular ion peak at *m/z*, 268 amu (MH+) in positive ion mode, whereas the Lansoprazole (Fig-6), displayed protonated molecular ion at m/z 370. Thus the degradation impurity has 102 amu less than the molecular ion of Lansoprazole indicating that loss of two carbon atoms, five hydrogen atoms, one oxygen atom and three fluorine atoms in the molecule.

IR absorption spectral data of degradation impurity (Table-2) also supporting that formation of one carbonyl functional group and thione (-C=S) functional group. The IR (KBr) spectral data of the degradation impurity was compared with those of Lansoprazole in Table-2. IR absorption bands for this impurity (cm−1) are 2926 (aromatic C-H stretching), 2870 (aliphatic C-H stretching), 1722 (C=O stretching), 1578 (aromatic C=C stretching), 1184 (C=S stretching), 1093 (C-N stretching).

From the spectral data, the structure of this degradation impurity is characterized as 1-Methyl-10-thioxo-10H-4a,5,9b-triaza-indeno[2,1-a]inden-2-one with molecular formula $C_{14}H_9N_3OS$ and molecular weight 267.1.

Formation of degradation impurity

The degradation impurity formed in the presence of acid stress degradation is due to the formation of thioxo cyclic ring and the removal of trifluoro ethyl group. The probable degradation pathway is shown in Fig-8.

CONCLUSION

The major unknown acid degradation product was isolated by preparative LC and was characterized by using spectroscopic techniques namely NMR, HR-MS, ESI-MS and IR. This work demonstrated the practical utility of NMR, LC-ESI-MS in the efficient structural elucidation of the novel degradation products of bulk drug substances. Thus in case of impurities where the quantity of the isolated impurity is less, these techniques come to rescue and help in carrying out the structural elucidation efficiently.

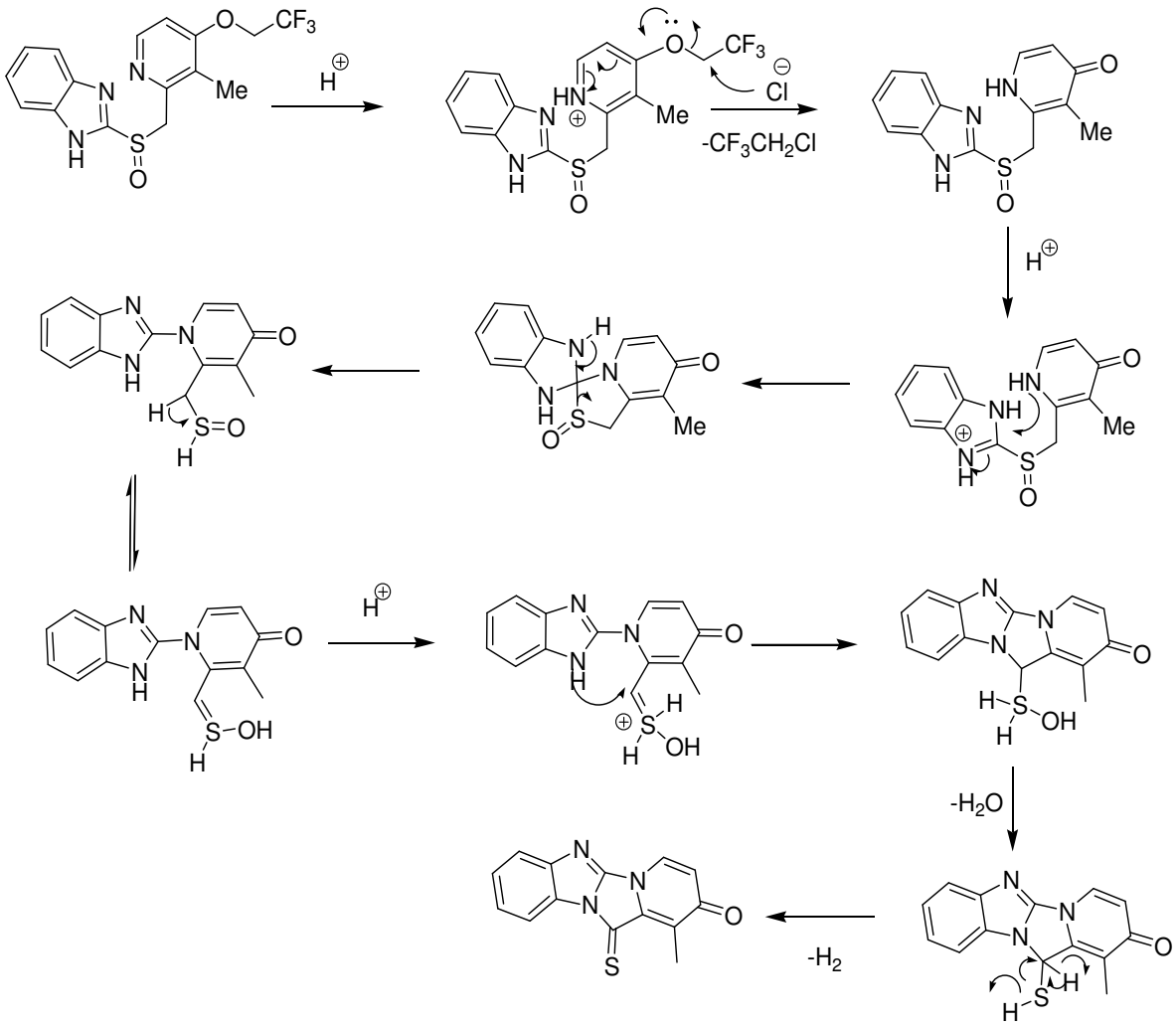


Fig.-8: Proposed degradation pathway for the degradation product

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