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# Isolation and identification of the major degradation product in lansoprazole capsule during stability studies

**Bhawana Negi*, Abhishek Miglani, Poonam Kumar**

**Research & Development, Analytical Formulation, Jubilant Organosys Ltd., D-12, Sector-59, Noida-201301, Uttar Pradesh, (INDIA)**

**E-mail: bhawana_negi@jubl.com**

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## ABSTRACT

The presence of unknown impurity of the order 0.2% was identified in the Lansoprazole Delayed Release Capsule using liquid chromatographic technique employing binary gradient system. A simple high-performance liquid-electrospray ionization mass spectrometric method has been developed for the identification of unknown impurity in Lansoprazole Delayed Release Capsule. The ESI-MS results obtained allowed us to propose possible structure for its fragmentations. Structure elucidation using nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy was facilitated by developed rapid preparative isolation method. The impurity was characterized as 1 *H* –benzimidazole-2-thiol. 

## KEYWORDS

Lansoprazole;
Impurity identification;
Impurity isolation;
Impurity characterization;
LC-MS-MS.

## INTRODUCTION

Lansoprazole, 2-[[[3-Methyl-4-(2, 2, 2-trifluoroethoxy)-2-pyridyl]-methyl] sulfinyl] benzimidazole is a gastric $H^+K^+$-ATPase (proton pump) inhibitor[1]. Lansoprazole belongs to the class α-pyridylmethylsulfinyl benzimidazole. It Binds covalently to parietal cell $H^+$ $K^+$-ATPase, rendering it nonfunctional and inhibiting the secretion of gastric acid[2]. Lansoprazole is used for the prevention and treatment of gastric acid related diseases. Several spectrophotometric methods for the determination of Lansoprazole were described earlier[3-6]. However very little information is available for the determination of its impurities[7-9].

During the analysis of samples of Lansoprazole DR Capsules, an unknown degradate was observed by HPLC at level 0.20% relative to Lansoprazole which is crossing the limit for individual unknown impurity of 0.1% as mentioned in USP monograph[10]. The requirement of identification & characterization of this impurity is extremely necessary to meet the astringent regulatory requirements & to ensure that safety of drug substance is not compromised by presence of toxic impurities[11]. Formation of 3 degradants Sulfone, N-Oxide and sulfide impurity in Lansoprazole is already reported in USP monograph. The chromatographic retention of the degradate observed in the Delayed Release Capsule formulation did not correspond to any of these three known degradates. Chromatographic conditions mentioned in United State Pharmacopoeia (USP) is not compatible with mass spectrometric detection. In order to get better ionization the method has been modified for the present investigation.

Present paper describes identification, isolation and structural elucidation of unknown impurity

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## EXPERIMENTAL

### Samples and chemicals

Samples of an Experimental Formulation that contain pharmaceutical grade granules of Lansoprazole Delayed-Release Capsule were obtained from formulation R&D Department, Jubilant Organosys Ltd., Noida, India. Impurity sulfone, N-Oxide and Sulfide were synthesized in the laboratory after identification by HPLC and determination of molecular weight by LC-MS. LC-grade water was prepared by purifying distilled water with a Milli-Q water purification system from Millipore (Malsheim, France). HPLC grade Acetonitrile was purchased from Qualigens, Ammonium Formate was purchased from Merck India Limited. DimethySulfoxide-d6 (for NMR) was purchased from Aldrich Chemical Co., USA

### High performance liquid chromatography (HPLC)

Lansoprazole samples were analyzed on a waters Alliance 2690 HPLC equipped with Waters 2487 UV detector. Kromasil C18 Column 150mm x 4.6mm, 5µm (AKZONOBEL, Brewster NY, USA) was used for Chromatographic separation. Flow rate was maintained at 0.8 mL /min with UV detection at 285 nm. Mobile phase A used for separation was water & Mobile phase B consist of Water-Acetonitrile-TEA (200:800:5, v/v/v) and pH of mobile phase B adjusted to 7.0 with dilute OPA.Gradient program was used and method was able to detect all the impurities. Following gradient was applied % Mobile Phase B (time, min): 10(0), 80(40), 80(50), 10(51), 10(60)

### Liquid chromatography tandem mass spectrometry

The MS/MS studies were carried on Q-Tof Micromass System (Waters). The HPLC consisted of Waters Alliance 2690 series quaternary gradient pump with a degasser an autosampler & column oven interfaced with Q-Tof Micromass Spectrometer via an ESI Probe. HPLC effluent was introduced into Electro spray ionization (ESI) source of mass spectrometer. Capillary voltage was maintained at 3000 V, Sample cone voltage at 25 V and Extraction cone voltage at 2V. Nitrogen was used as both desolvation and nebulizing gas. Cone gas flow maintained at 50 L/hr and desolvation gas flow maintained at 500L/hr.

MS/MS studies were carried out by maintaining Collision Energy at 20 and mass range 100- 1000 amu. Negative ionization was also performed by switching the polarity of capillary voltage to -3000 V.

A second method was developed using mobile phase compatible with LC/MS instrument. Chromatographic conditions were same as above method except that mobile phase B was composed of Ammonium Formate (10mM)-Acetonitrile (200:800, v/v)

### Preparative liquid chromatography method

The samples of Lansoprazole Capsules were sonicated in Acetonitrile and then filtered under vacuum and filtrate was concentrated on Rotavapor (BUCHI Labortechnik AG Flawil, Switzerland). Impurity was isolated from the crude sample using Agilent 1200 Series Auto purification System Consisting of binary gradient pump, Multiwavelength detector, sample manager and fraction collector (Agilent Technologies Waldbrom, Germany). A waters symmetry C18 column 300 mmx19 mm i.d, particle size 7µ was used for the separation. Mobile phase was consisted of a mixture of Ammonium formate (10mM)-Aceonitrile (80:20, v/v). Detection was monitored at 285 nm.

### NMR

$^{1}H$ and $^{13}C$ spectra of isolated impurity were recorded on Bruker 400 HZ instrument. The $^{1}H$ and $^{13}C$ chemical shift values were recorded and δ ppm scale relative to DMSO

### IR spectroscopy

The IR Spectra of isolated impurity was recorded in the solid state as KBr powder disc using Perkin Elmer FT-IR spectrometer.

## RESULT AND DISCUSSION

### Detection of impurity by HPLC and LC-MS

HPLC analysis of sample of lansorazole capsule was carried out using the method discussed in section 2.2. Apart from the principle peak (eluting at 25.7 min) one unknown impurity was detected at retention time

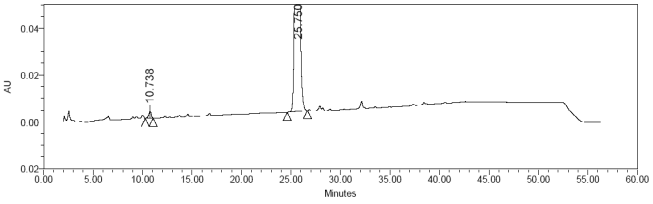


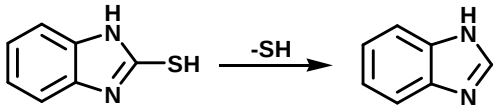

**Figure 1 : HPLC chromatogram of stability sample of lansoprazole delayed release capsule**

10.7min, respectively. A typical chromatograph shown in figure 1.

## LC/MS/MS analysis

Prior to characterization work on this unknown impurity LC/MS/MS data for Lansoprazole drug molecule was generated. Mass spectrum of Lansoprazole show a protonated molecular ion peak $[M+H]^+$ at m/z 370. The MS/MS spectrum for the parent Lansoprazole molecule show fragment peaks at m/z 252, 150 and 118 amu. Fragment of 252 can be attributed to the loss of benzimidazole moiety.

Unknown impurity exhibited protonated molecular ion peak having mass to charge ratio 150 amu. Daughter ion MS/MS spectrum of the parent ion of the impurity gave fragment of m/z 118. Formation of daughter ion attributed to the loss of SH from the parent ion peak. Mechanism for this fragmentation can be rationalized from the structure shown in figure 2.




**Formula Weight = 150.202** **Formula Weight = 118.136**

**Figure 2 : Mechanism for the formation of fragment ion from impurity**

## Isolation of impurities by preparative HPLC

A newly developed isocratic reverse phase chromatographic method described in section 2.4. was used for isolating unknown impurity. The retention time for unknown impurity and lansoprazole were observed at 4 and 7 min., respectively. The collected fractions were combined and concentrated on rotavapor and dried under high vacuum using lyophilizer. Chromatographic purity of the isolated impurity sample was determined by HPLC and found to be 99 %, respectively as shown in figure 3. This sample was used further for spectroscopic studies.

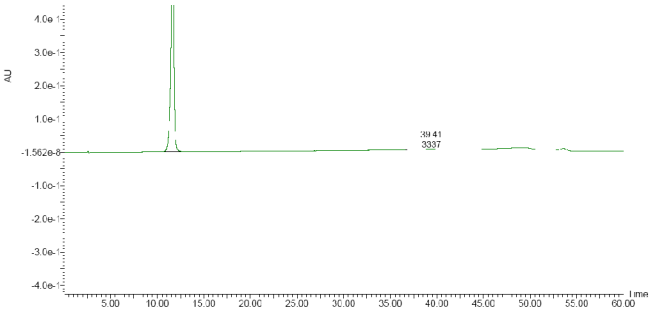



**Figure 3 : HPLC chromatogram of isolated impurity**

## Structure elucidation of impurity

The isolated impurity was subjected for structural analysis using MS, LCMS/MS, $^1H$, $^{13}C$ NMR & FT-IR spectroscopic methods.

The +ve ES-MS spectrum of the impurity showed peak at m/z 151 corresponding to the adduct ion $(M+H)^+$. The MS/MS spectrum of the impurity displayed daughter ion at m/z 118 amu (Figure 4.). Fragment of 150 & 118 were also obtained with the Lansoprazole suggesting the structural similarity between impurity and Lansoprazole. Further the-ve ES-MS spectrum showed peak at m/z 149 corresponding to $(M-H)^-$ (Figure 5).

The ESI mass spectrum of impurity exhibited a molecular ion peak at m/z 151 amu. In the FT-IR spectrum, a characteristic absorption band was appeared

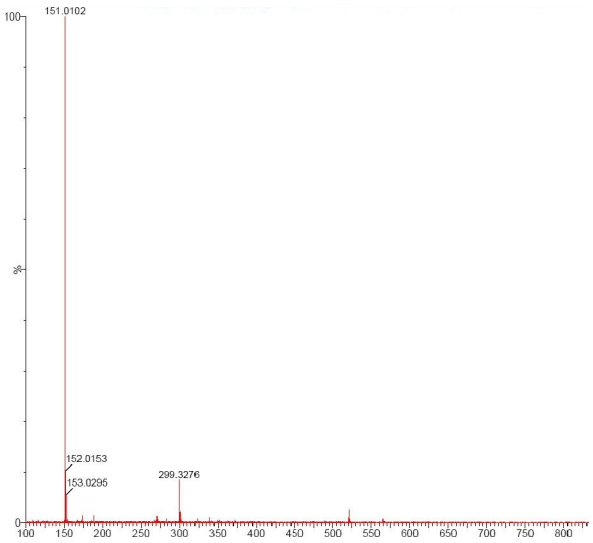



**Figure 4(a) : Mass spectrum of impurity in ES+ve mode**

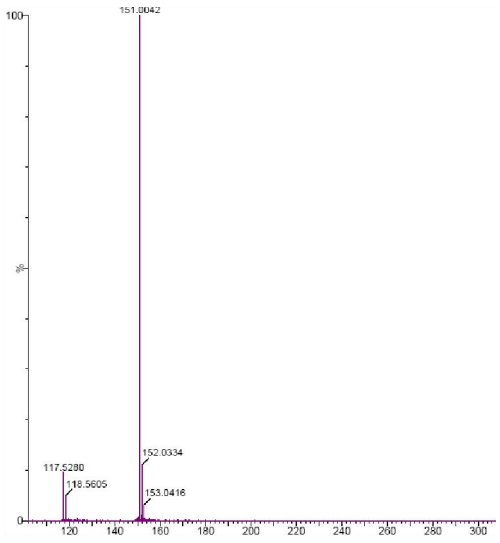



**Figure 4(b) : MS/MS spectrum of impurity in ES+ve mode**

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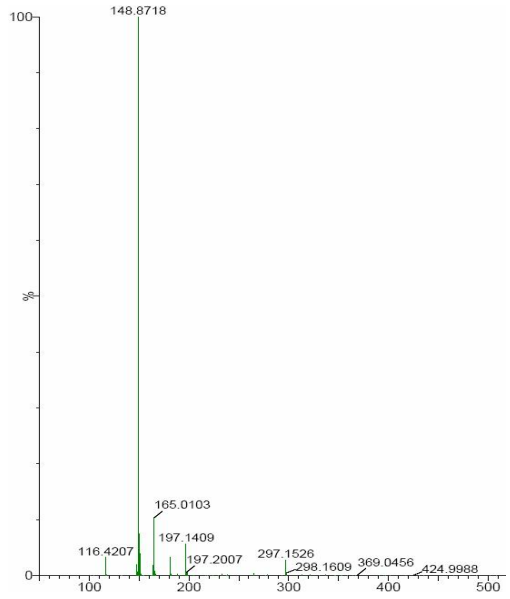


**Figure 5 : Mass spectrum of impurity in ES-ve mode**

at 3153 $cm^{-1}$ for > N-H stretching vibration. In the $^1H$ proton spectrum of impurity, in DMSO –NH proton appeared at 12.5 ppm and 4 aromatic protons appeared in the region (7.0-7.1 ppm) and –SH proton appeared at 3.3 ppm. Detailed description is given in TABLE 1. Fragment of 118 amu was obtained with the Impurity with the loss of 33 amu from the parent ion showing the loss of SH group in the impurity. Taken together all these data support the structure of impurity that was proposed on the basis of MS/MS data.

Based on the above spectral data the molecular formula of impurity was confirmed and the corresponding structure was characterized as 1 H –benzimidazole-2-thiol.

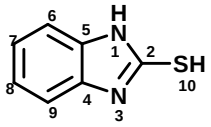


**Figure 6 : Structure of impurity 1*H*-benzimidazole-2-thiol**

**TABLE 1 : NMR spectral assignments for impurity**

| Position | No. of Protons | Proton Chemical Shift | 13C Chemical Shift |
|---|---|---|---|
| 1 | 1 | 12.5 | - |
| 2 | - | - | 168.5 |
| 3 | - | - | - |
| 4 | - | - | 132.6 |
| 5 | - | - | 132.6 |
| 6 | 1 | 7.1 | 109.9 |
| 7 | 1 | 7.0 | 122.7 |
| 8 | 1 | 7.0 | 122.7 |
| 9 | 1 | 7.1 | 109.9 |
| 10 | 1 | 3.3 | - |

**Refer the structural formula for numbering (Fig. 6).**

## CONCLUSION

In this study impurity profile of Lansoprazole has been carried out by LC/MS and LC/MS/MS. Preliminary structure assignments for the unknown impurity was made on the basis of mass spectral data. The complete characterization of the compound was carried out by various spectroscopic studies after preparative chromatographic isolation.

## ACKNOWLEDGEMENTS

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## REFERENCES

**[1]** Joel G.Hardman, Lee E.Limbird, Goodman & Gilman's; 'The Pharmacological Basic of Therapeutics', 10th Ed., McGraw-Hill Co., USA, 1007-1009 **(2001)**.

**[2]** Erdinc Naseri, Aydan Yenisehirli; Eur.J.Pharmacol., **531**, 226-231 **(2006)**.

**[3]** M.D.Karol, G.R.Granneman, K.Alexandr; J.Chrom. B, **668**, 182-186 **(1995)**.

**[4]** M.Miura, H.Tada, T.Suzuki; J.Chrom.B, **804**, 389-395 **(2004)**.

**[5]** B.Prasanna Kumar Reddy, Y.Ramamjaneya Reddy, D.Ramanchandran; E-Journal of Chem., **6**, 489-494 **(2009)**.

**[6]** Kaili, Jiajie He, Xiaoya Ding; PPD-8551 Reserchway, Suite 92, Middleton, W153562, http://www.wayne.way@sial.com.

**[7]** R.Petkvoska, C.Cornett, A.Dimitrovska; J.Liquid Chrom.& Related Tech., **31**, 2159-2173 **(2008)**.

**[8]** J.Selenka, S.Duff, J.He, K.Li, P.Sunthankar, X.Ding; http://www.aapsj.org/abstracts/AM-2007/AAPS2007-003647.pdf.

**[9]** Zeinaf Abdelaziz El-Sherif, Afaf Osman Mohamed, Mohamed Galal El-Bardicy, Mohamed El-Tarras; Chemical & Pharmaceutical Bulletin, **54(6)**, 814-818.

**[10]** United State Pharmacopeia, **31**, 2502-2504.

**[11]** ICH topic Q1A ($R_2$); Stability Testing of New Drug Substances and Products, Proceedings of the International Conference on Harmonization, London, **(2003)**.