Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 10, 2014

Original Article

ISOLATION AND CHARACTERISATION OF NOVEL IMPURITY OF LANSOPRAZOLE FORMED IN THERMAL STRESS CONDITION

SATYANARAYANA BATTU*, VASUDEV POTTABATHINI

Department of Chemistry, University College of Science, Osmania University, Hyderabad 500007
Email: satyambchem@yahoo.co.in

Received: 03 Aug 2014 Revised and Accepted: 05 Sep 2014

ABSTRACT

Objective: The aim of the research work is to study the degradation of Lansoprazole in stress condition which is a proton pump inhibitor and controls the production of gastric acid in stomach. Thermal stress condition has been used to check the stability of the compound, in which one new degradation product was generated.

Methods: The USP method for the identification of related compounds of Lansoprazole shows three known impurities N-Oxide impurity, Sulphone impurity and sulphide impurity. New degradation product formed in stress condition has been separated from the drug product and other known impurities by using Clarity Oligo RP column (250 mm x 4.6 mm, 5µ) and 10 mM Ammonium Acetate -Acetonitrile as a mobile phase. Same method has been scaled up on mass based preparative HPLC for the isolation of new degradation product.

Results: The isolated impurity was structurally elucidated with ^{1}H NMR, ^{13}C NMR, HMBC, HSQC and HRMS. From the characterization studies it was found that novel impurity has 164.04 molecular weight with molecular formula $C_8H_8N_2S$. The degradation product's structure was matched with 2-(Methylthio)-1H-benzo[d]imidazole per recorded analysis data.

Conclusion: Isolated impurity was found to be novel and not reported in the literature. This method can be used for the degradation study of Lansoprazole and purification of novel impurity from drug and other degradation products

Keywords: Lansoprazole, Thermal degradation, Structural elucidation, Preparative HPLC.

INTRODUCTION

The identification and qualification of impurities or degradants in pharmaceuticals is critical in terms of product efficacy and drug safety. Regulatory bodies, such as the U. S. Food and Drug Administration (FDA), and the International Conference on Harmonization (ICH) have established clear and rigorous guidelines for setting thresholds for the reporting, identification, and qualification of impurities in drug substances and drug products. Stress testing is an important aspect of the drug development process. Appropriate stress testing can greatly assist the elucidation of forced degradation pathways. Recent efforts by the ICH with regard to stability and photo stability testing have brought increased regulatory scrutiny of impurities and the need to identify and qualify impurities at lower levels [1].

Controlling degradation-related impurities involves identifying which of the potential degradation products found during stress testing actually form in either the drug substance or product under long-term or accelerated storage conditions and then selecting the appropriate countermeasures to minimize the impurities or degradants. An impurity profiling study of forced degradation samples of Lansoprazole drug substance illustrates the identification process and its potential impact on pharmaceutical development [2]. Lansoprazole is a substituted benzimidazole, named as(*RS*)-2-({[3- methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methyl}sulfinyl)-1*H*-benzimidazole. Its empirical formulais $C_{16}H_{14}F_3N_3O_2S$with a molecular weight of 369.36. Lansoprazole and its USP Impurities, Reported impurity and newly formed Thermal degradant impurities were shown in Fig.1. Lansoprazole is used to treat and prevent stomach intestinal ulcers, erosive esophagit is (damage to the esophagus from stomach acid), and other conditions involving excessive stomach acid such as Zollinger-Ellison syndrome [3].

In the literature, limited LC methods were reported for the determination of the assay and related substances of LAN. Some analytical assay methods were reported for the estimation of individual and combinations with other drugs in human plasma and dosage forms [4–12]. One chiral method and chemo metric approach by HPLC is available to separate known impurities of Lansoprazole, but has not proven the stability-indicating nature [13-14]. Some research work was done on the synthesis and characterisation of impurities formed during the forced degradation of LANS [15-16]. The present study is on impurities formed during the forced degradation under thermal stress conditions.

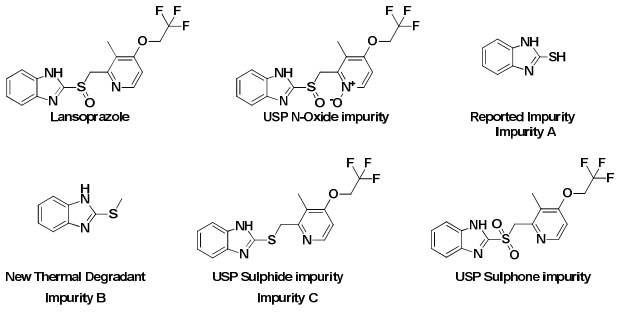


Fig. 1: Chemical Structures of Lansoprazole and its USP Impurities, Reported impurity and newly formed Thermal degradant

MATERIALS AND METHODS

The investigated sample of lansoprazole and its USP impurities were procured from Nosch Labs Private Limited, India. Solvents and buffer used for analysis were HPLC grade acetonitrile and methanol (Rankem), Ammonium acetate (Fisher Scientific-Qualigens) and Water used was Milli-Q grade.

Equipment

Mass mediated high performance liquid chromatography

A Mass mediated preparative HPLC equipped with waters pump module 2545, UV detector module-2996 and mass detector module 3100, Sample manager module-2767, Mass lynx data handling system was

used. This system was equipped with both analytical port and preparative port. Mass capillary voltage was maintained 3 kv, Source temperature 150 ^{0}C and disolvation temperature 350 ^{0}C for the proper ionisation. 0.1 % formic acid is used in Water: Methanol (90:10) as a makeup solvent to the mass detector through splitter.

Mass mediated analytical HPLC parameters

Column: Clarity 5 µ Oligo RP (250 x 4.6)

Mobile phase: 10 mM Ammonium Acetate (A): Acetonitrile (B)

T/ % of B: 0.01/10, 5/10, 5.1/35, 25/35, 25.1/90, 30/90, 30.1/10, 35/10

Diluent: Mobile phase

UV Detection: 285 nm

HRMS (High Resolution Mass Spectrometry)

Sample was analysed on the waters micro mass Q-TOF equipped with ESI ion source. Sample was analysed in positive mode. Caffeine (m/z: 194.080383 Da) was used as internal standard to calibrate the mass range and mass accuracy. Data was acquired in positive mode using Masslynx software.

Nuclear magnetic resonance spectroscopy (^{1}H&^{13}C -NMR, HMBC, HSQC)

The ^{1}H and ^{13}C NMR spectra of thermal degraded Impurity were recorded in DMSO-d_6 at 400 MHz, Bruker 400MHz Advance NMR Spectrometer. The ^{1}H and ^{13}C chemical shifts are reported on δ scale in ppm, relative to TMS (δ 0.00 ppm) and DMSO-d_6 (δ 39.50 ppm) as internal standards respectively.

Thermal stress method

Thermal degradation studies were carried out as per the guidelines of ICH. 100 mg of standard drug heated at 105 ^{o} C for 8 h to study thermal degradation. For analytical study thermally exposed sample was dissolved in methanol and diluted with mobile phase and 10 µl injected in to the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

Identification of new thermal degradation product

After thermal degradation the crude sample was injected into the analytical column for the separation of degradation products from the drug peak. To obtain the baseline separation different mobile phases has been used like ammonium formate, ammonium acetate, Trifluro acetic acid, formic acid and acetic acid. Various columns were screened to check the separation and symmetrical peak shape like YMC ODS, YMC Triart, Sunfire, X-terra, X-bridge, Agilent zorbax CN, Zorbax phenyl and Clarity Oligo. Finally desired separation was achieved using the 10 mM ammonium acetate and acetonitrile as a mobile phase and Clarity Oligo column (250 x 4.6 mm, 5µ). In the developed method, the chromatogram shows Peak-2 (RT 8.40, 19.95%) as a novel degradant having different mass than reported impurities in the literature. Which indicates that peak-2 is a novel thermal degradant formed in this degradation procedure. The details of the degradation products retention time and molecular weights has been compiled in the in the below table-1.

Table 1: Degradation products formed on Thermal stress condition

S. No.	Peak Label	Retention time	Area%	Observed mass	Exact mass	About the peak
1	Peak -1	6.45	12.02	149.15 (M-H)	150.20	Reported Impurity-A
2	Peak-2	8.40	19.95	163.20 (M-H)	164.04	NovelThermal impurity-B
3	Peak-3	16.62	24.33	368.29 (M-H)	369.08	Drug Lansoprazole
4	Peak-4	24.85	22.48	352.30 (M-H)	353.08	USP Impurity-C

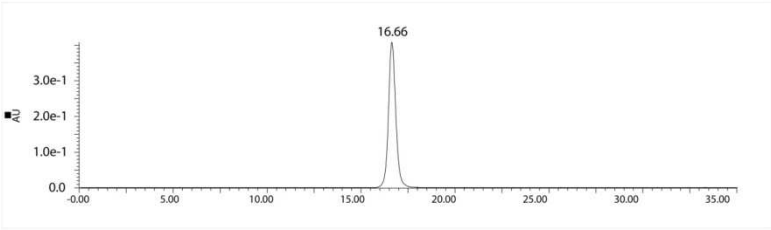


Fig. 2: Standard chromatogram of Lansoprazole

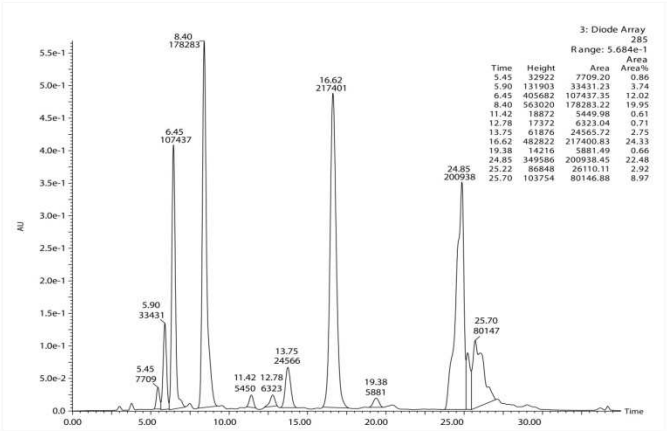


Fig. 3: Chromatogram of Lansoprazole after degradation

Isolation of thermal degradation product

This degradation study clearly indicates that the peak observed at 8.40 min retention time and having mass m/z 163.20 (M-H) was new entity in thermal degradation and remaining peaks were matching with the already reported impurities. To separate this novel impurity formed in stressed thermal condition we scaled up the analytical method to preparative scale. Using the same mobile phase and column having dimension (250 x 19 mm, 5µ) and a flow rate 19 ml min^{-1}degradation product has been isolated. In mass based preparative system splitter has been used after the column through which flow has been spiltted in the ratio 1000:1. The one part of the flow is passed through mass detector by diluting with make pump using 1 ml min^{-1} flow rate. For the proper ionisation of the desired impurity 0.1 % formic acid has been used as it is more volatile and enhances the ionisation. The delay time of the fraction collector has been investigated by using dye at same flow rate and the same parameters have been given as a input to get the good recovery. The crude sample was diluted with mobile phase and injected into the preparative column in three consecutive injections. The fractions have been collected on the basis of mass threshold parameters of total ion chromatogram. After completion of purification collected all fractions of mass 163.20 (M-H) pooled together and lyophilized to get free solid.

Structure elucidation of thermal degradation product

Compound m/z: 164.04 obtained from lyophilisation characterized by using HRMS, NMR (^{1}H-NMR, ^{13} C NMR, HMBC and HSQC).

High resolution mass spectrometry

Sample was analysed on the waters micro mass q-TOF equipped with ESI ion source. Sample was analysed in positive mode. Caffeine (m/z: 194.080383 Da) was used as internal standard to calibrate the mass range and mass accuracy. Data was acquired in positive mode using Masslynx software.

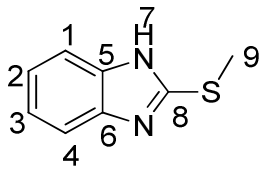
From the mass spectrum it was showing

1. Monoisotopic mass with even electron ions (m/z: 165.0463(M+H))
2. Elements observed: C: 0-8 H: 0-9 N: 0-2 S: 0-1
3. Molecular Formula: C8 H9 N2 S (M+H)

From information provided by HRMS report it is matching with expected structure of thermal degradant.

Fig. 4: Mass elemental composition report of thermal degradant.

Nuclear Magnetic Resonance (^{1}H&^{13}C -NMR, HMBC, HSQC):



Thermal degradant

Table 2: NMR chemical shift values for Thermal degradation product

Position	1 H	^{1}HChemical shift in PPM	^{13}C Chemical shift in PPM
1	1H	7.7	112.971
2	1H	7.5	125.052
3	1H	7.5	125.052
4	1H	7.7	112.971
5	-	-	132.489
6	-	-	132.489
7	1H	10.6	-
8	-	-	152.689
9	3H	2.9	14.4

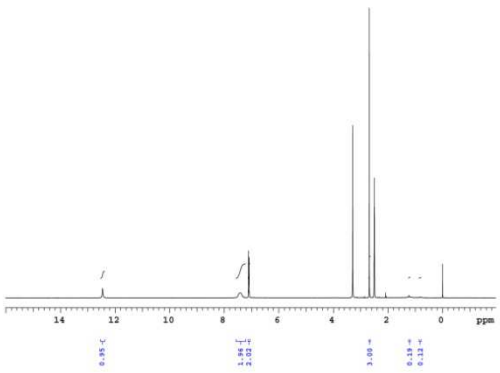


Fig. 5: ^{1}H NMR spectrum of degradation product in DMSO

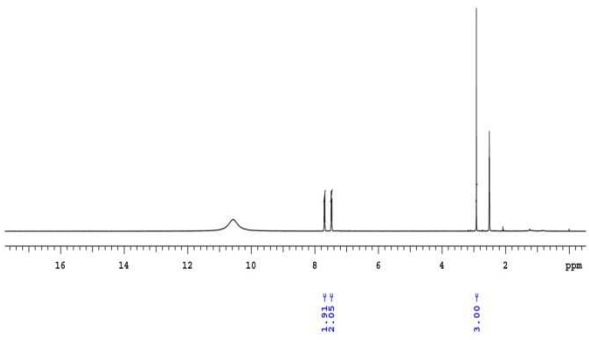


Fig. 6: ^{1}H NMR Spectrum of degradation product in DMSO+TFA

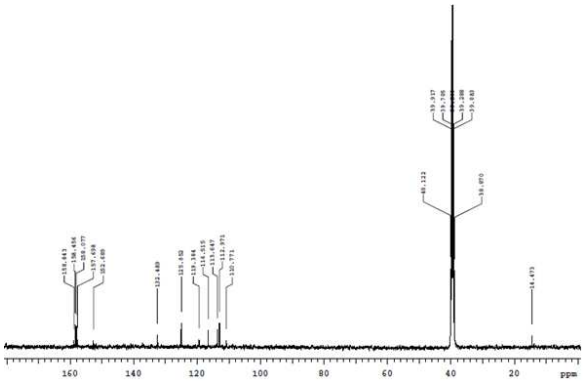


Fig. 7: ^{13}C NMR Spectrum, Extra signals due to DMSO+TFA

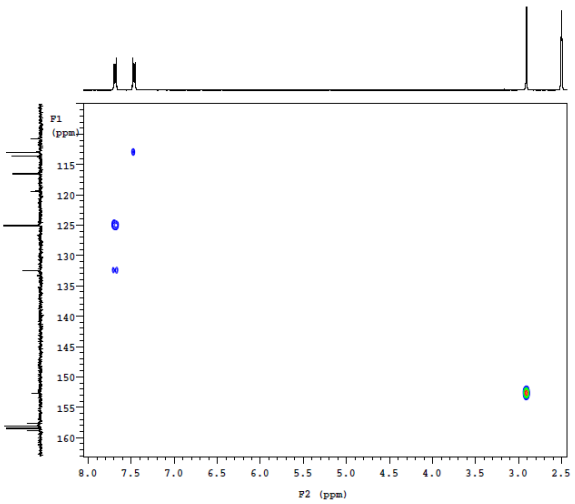


Fig. 8: HMBC data of degradation product

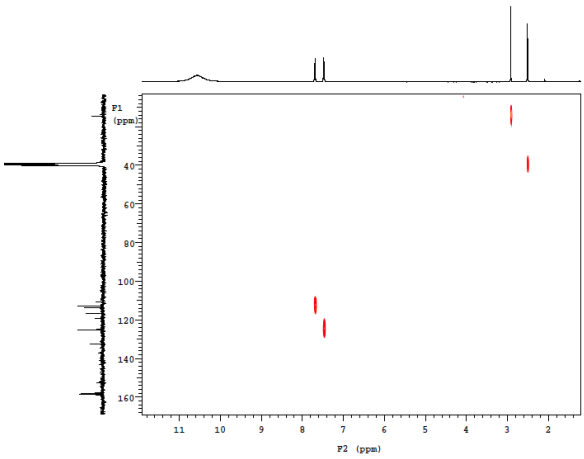


Fig. 9: HSQC report of degradation product

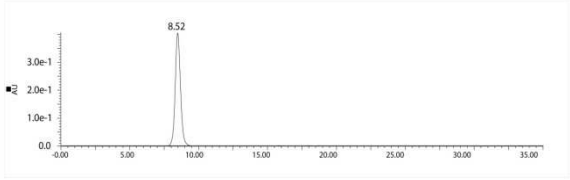


Fig. 10: Newly formed thermal degradation product purity chromatogram.

From the above information obtained from the HRMS and NMR spectral data it is clearly matching the assumed structure of thermal degradation product. The newly formed thermal degradation product was separated in this developed method from its USP impurities and Lansoprazole. Chromatogram of Lansoprazole, USP impurities and newly formed thermal impurity were shown below.

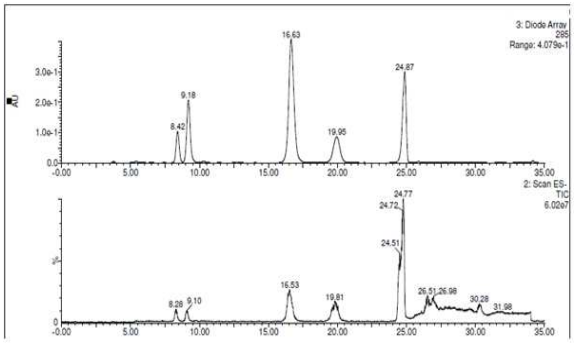


Fig. 11: Chromatogram of Lansoprazole, USP impurities and newly formed thermal impurity

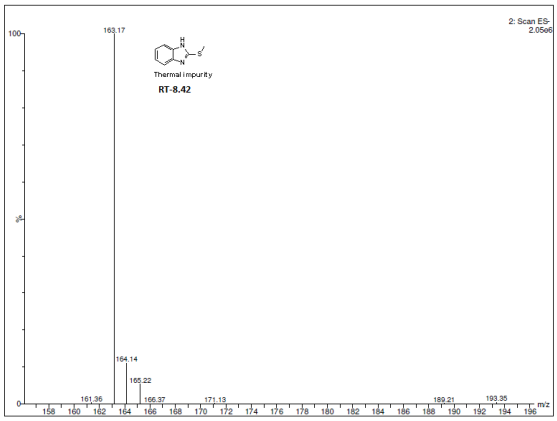


Fig. 12: Mass spectrum of newly formed thermal degradation product

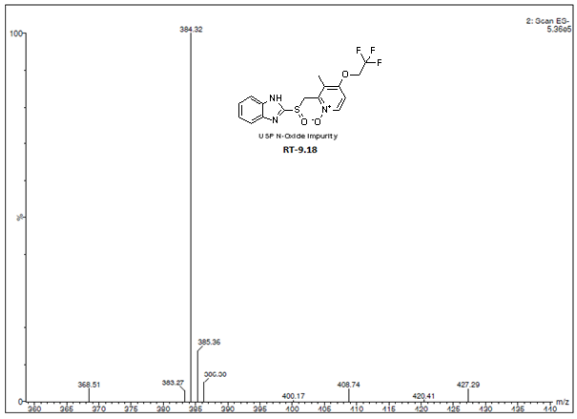


Fig. 13: Mass spectrum of USP N-Oxide impurity

Table 3: HSQC& HMBC Correlations

HSQC	HMBC
3H (2.9) 14.4	3H (2.9) 152.689 (Quaternary)
2H (7.7) 112.971	2H (7.7) 125.052, 132.489
2H (7.5) 125.052	2H (7.5) 112.971

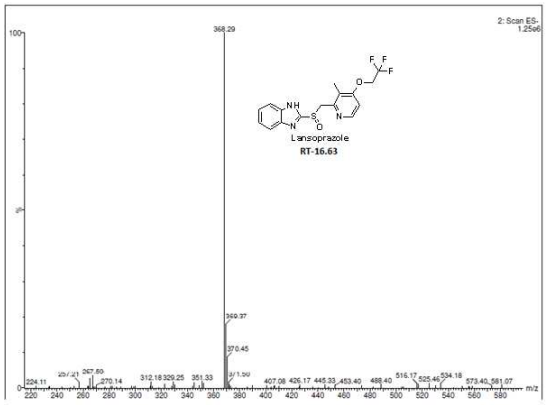


Fig. 14: Mass spectrum of Lansoprazole

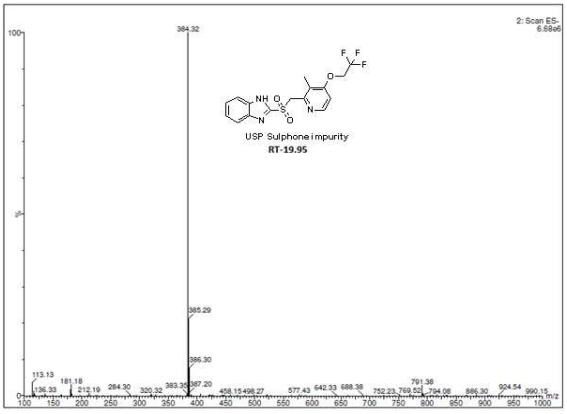


Fig. 15: Mass spectrum of USP Sulphone impurity

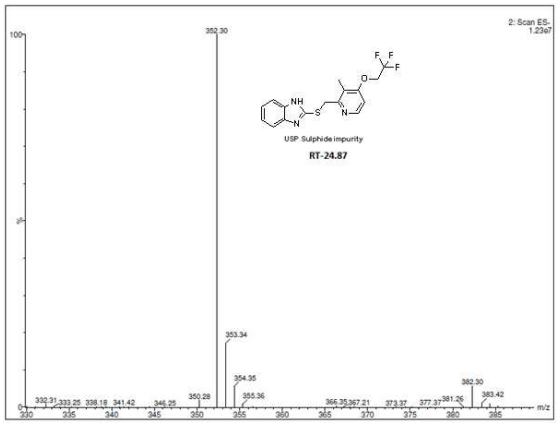


Fig. 16: Mass spectrum of USP Sulphide impurity

CONCLUSION

The fast, simple and sensitive method has been optimised for the separation of degradation product of Lansoprazole in thermally stressed condition. The thermal degraded impurity of Lansoprazole was identified and isolated using a mass mediated preparative HPLC system equipped analytical and preparative ports. Isolated degradant product was characterized by HRMS and NMR (^1H, ^{13}C and HMBC, HSQC) techniques. The HRMS and NMR spectral data of isolated product was confirmed to have a mass of 164.04 and with molecular formula of $C_8H_8N_2S$. It was further confirmed that the isolated thermal impurity from this method is different from all the reported impurities of Lansoprazole in the literature, having chemical name of 2-(Methylthio)-1H-benzo[d] imidazole.

CONFLICT OF INTERESTS

Declared None

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