Forced degradation studies of lansoprazole using LC-ESI HRMS and ^{1}H-NMR experiments: *in vitro* toxicity evaluation of major degradation products

G. Shankar[1], Roshan M. Borkar[1], Suresh Udutha[1], Lalitha Guntuku[2], VGM Naidu[2], Narayana Nagesh[3] and R. Srinivas[1]*

[1]*National Centre for Mass Spectrometry, CSIR-Indian Institute of Chemical Technology, Hyderabad, 500 007, India*

[2]*Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research, Balanagar, Hyderabad 500037, India*

[3]*CSIR-Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500007, India*

Keywords: Lansoprazole, degradation products, accurate mass measurements and *in vitro* toxicity

* Corresponding author :srini@iict.res.in (R. Srinivas)

sragampeta@yahoo.co.in

Telephone Number :+91-40-27193122

Fax Number : +91-40-27193156

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jms.3949

Abstract

Regulatory agencies from all over the world have set up stringent guidelines with regard to drug degradation products due to their toxic effects or carcinogenicity. Lansoprazole, a proton-pump inhibitor, was subjected to forced degradation studies as per ICH guidelines Q1A (R2). The drug was found to degrade under acidic, basic, neutral hydrolysis and oxidative stress conditions, whereas it was found to be stable under thermal and photolytic conditions. The chromatographic separation of the drug and its degradation products were achieved on a Hiber Purospher, C18 (250 X 4.6mm, 5µ) column using 10 mM ammonium acetate and acetonitrile as a mobile phase in a gradient elution mode at a flow rate of 1.0 mL/min. The eight degradation products (DP1-8) were identified and characterized by UPLC/ESI/HRMS with in-source CID experiments combined with accurate mass measurements. DP-1, DP-2 and DP-3 were formed in acidic, DP-4 in basic, DP-5 in neutral and DP-1, DP-6, DP-7 and DP-8 were in oxidation stress condition Among eight degradation products, five were hitherto unknown degradation products. In addition, one of the major degradation products, DP-2 was isolated by using semi preparative HPLC and other two, DP-6 and DP-7 were synthesized. The cytotoxic effect of these degradation products (DP-2, DP-6 and DP-7) were tested on normal human cells such as HEK 293 (embryonic kidney cells) and RWPE-1(normal prostate epithelial cells) by MTT assay. From the results of cytotoxicity, it was found that lansoprazole as well as its degradation products (DP-2, DP-6 and DP-7) were nontoxic up to 50 µM concentrations and the latter showed slightly higher cytotoxicity when compared with that of lansoprazole. DNA binding studies using spectroscopic techniques indicate that DP-2, DP-6 and DP-7 molecules interact with ctDNA and may bind to its surface.

1.Introduction

Stress degradation studies are conducted on the drug substances and drug products under more severe conditions than accelerated stability studies. [1] All drug substances and drug products have a tendency to degrade over the period of time under the influence of catalytic external and internal factor such as heat, moisture, pH, oxidants, light, etc. The main purpose of the stability studies is to establish the intrinsic stability of drug substance and to provide more comprehensive information on the drug degradation pathway by using highly sensitive and sophisticated analytical tools. [2-4] These studies will help to evaluate the time up to which any drug molecule or formulation would retain the quality safety, efficacy specification. Characterization of the trace level degradation products has become possible with the help of sophisticated analytical tools. Structure elucidation and evaluation of the toxicity of the degradation product is important from safety point of view. Severe adverse effects of the drug degradation products have been reported for the degradation product of tetracycline and aminopencillins. [5-6] Recently, we have reported in *vitro* toxicity of degradation products of rabeprazole. [7] It showed more than 50% of cell inhibition on HepG2 and PANC-1 cell lines. DNA binding studies indicated that the degradation products bind to the surface of double stranded DNA and stabilize the DNA complex.

Due to toxic effect or carcinogenicity of degradant, stringent reporting has been setup by several regulatory agencies from all over the world. Stability testing protocol was different from region to region. It differs from one manufacture to another, and introduction of International Conference on Harmonization (ICH) guideline brought protocols in a harmonized way. ICH Q3 guideline includes identification and qualification thresholds of degradation products of drugs. [8-9] The guidelineQ1A (R2) highlighted the purposes of stress testing and outlined a broad protocol. However, features were also covered in ICH guidelines Q1B [10], Q2 (R1) [1], Q3A [8], Q3B [9] and Q5C [11]. Other guidelines such as the World Health

Organization [12], United States Food and Drug Administration [13], European Medicines Agency [14] mention stress-testing requirements. e.g., US, Indian and Chinese pharmacopoeias, have recommended force degradation studies, whereas, British and European pharmacopoeias have not mentioned anything related to stress studies. The identified and characterized degradation products can be subjected to toxicity studies for risk assessment of potentially genotoxic degradation products.

Lansoprazole, ((RS)-2-([3-methyl-4-(2, 2, 2-trifluoroethoxy) pyridine-2yl] methylsulfinyl)-1H benzo[d]imidazole) is a proton-pump inhibitor which inhibits stomach's production of gastric acids. It is used to treat and prevent ulcer, Zollinger-Ellison syndrome and erosive esophagitis. Few HPLC methods have been reported for the determination of lansoprazole in plasma [15-16] and quantification of (R)-(+) - and (S)-(-)-lansoprazole enantiomers and related impurities [17]. Few studies have been reported on the characterization of acidic [18] and hydrolytic degradation products. [19] However, no study exists on systematic forced degradation behavior of lansoprazole under the influence of stress conditions as per ICH guidelines. Hence the purpose of this study is to identify and characterize the degradation products of lansoprazole using LC/ESI/HRMS and ^{1}H-NMR and to evaluate *in vitro* toxicities of major degradation products. In the present study, among eight degradation products a total of five hitherto unknown degradation products were identified and characterized. Degradation products, DP-1, DP-2 and DP-5 were reported earlier. [18-19] This study may help to evaluate the quality of drug product which are on the edge of expiration. Degradation products may have potential genotoxicity and leads to severe toxic effects.

2. Experimental

2.1 Chemicals and reagents

Lansoprazole (LAN), dimethyl sulfoxide-d6 (DMSO-d6), methanol-d4, tris hydrochloride (Tris-HCl), 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide and Deoxyribonucleic acid from calf thymus (ctDNA) was purchased from Sigma Aldrich, India.HPLC Grade acetonitrile (ACN) and methanol (MeOH) were purchased from Merck, India. HPLC grade water was prepared by filtrating through a Millipore Milli-Q- plus system (Millipore, Milford, MA, USA). Ammonium acetate of HPLC grade, ethyl acetate and sodium bicarbonate were purchased from Finar Chemicals Pvt. Ltd. (Ahmedabad, India). All analytical grade reagents, formic acid, sodium hydroxide, hydrochloric acid, dichloromethane (DCM)and 30% hydrogen peroxide were purchased from Merck (Mumbai, India).

2.2 Instrumentation

2.2.1. Liquid Chromatography-Mass spectrometry

Analysis was carried out on a U-HPLC instrument (Thermo Scientific Accela, Germany) equipped with a quaternary pump, a de-gasser, a diode-array detector, an auto sampler and a column compartment. Mass spectrometric analysis was carried out on an Orbitrap high-resolution mass spectrometer (Exactive Thermo Scientific, Germany) equipped with a heated electrospray ionization (ESI) source. The data acquisition was under the control of Xcalibur software.

2.2.2 Preparative HPLC

Isolation was carried out on a semi preparative HPLC instrument (GILSON, USA) equipped with a binary pump (331 and 332), a de-gasser, a diode-array detector (157 UV-VIS), a liquid handler (GX-241) and a column compartment. The data acquisition was under the control of TRILUTION LC software.

2.3. Stressed degradation conditions

Forced degradation studies of lansoprazole were carried out on the bulk drug as per ICH guidelines Q1A (R2). The drug solutions were prepared in 1.0mg/ml concentration for all the stressed reactions. Acidic hydrolysis of the drug was conducted in 0.01N HCl at room temperature for 15 min. Basic and neutral (H_2O: ACN, 50:50) hydrolysis were performed by refluxing drug in 2N NaOH and water at 80°c for 72 h and 48 h, respectively. The oxidative degradation study was conducted using 2% H_2O_2 at room temperature for 30min. Photolytic studies were performed by exposing solid and solution of the drug sample to 1.2×10^6 lux h of fluorescent light and 200W h/m^2 UV light in a photostability chamber. For thermal degradation study, the drug sample was sealed in glass vials and kept in a thermostatic block at 80°C for 1week. All stressed samples were kept in a refrigerator at 4°C until analysis.

2.4. Sample preparation

All the degradation samples were collected and neutralized. The collected samples were diluted ten times with the mobile phase and filtered through 0.22 µm membrane syringe filter before LC/ESI/MS analysis.

2.5. Analysis of stressed samples

2.5.1. Method development

The LC method was optimized for the separation of the drug and its degradation products using Hiber Purospher, C18 (250 X 4.6mm, 5µ) (Merck Lichro, Switzerland) column with a mobile phase composed of 10 mM ammonium acetate(A) and ACN(B). Initially, several gradient conditions were tried using MeOH/ACN with various pH buffers in different proportion. Finally, the mobile phase with a combination of 10 mM ammonium acetate and ACN gives better separation and good peak shape for lansoprazole and its degradation products (**DP 1-8**). The linear gradient programme as follows: (Tmin / % proportion of solvent B): $_{0\text{-}5}$/10, $_{5\text{-}7}$/30, $_{7\text{-}14}$/ 40, $_{14\text{-}20}$/45, $_{20\text{-}30}$/ 45, $_{30\text{-}35}$/ 50, $_{35\text{-}37}$/70, $_{37\text{-}38}$/10, $_{38\text{-}40}$/10. The column temperature, flow rate, injection volume, and detector wavelength were at 30 °C, 1.0

ml/min, 20.0 µl, and 285 nm, respectively. Samples analysis was performed in the ESI positive mode in the mass range from 50 to 600.The typical operating source conditions for MS scan were optimized as follows such as sheath gas flow rate 70; aux gas flow rate 32; spray voltage 3.50 kV; capillary temperature 380 °C; capillary voltage 25.00 V; tube lens voltage 100.0 V; skimmer voltage 18.00 V and heater or source temperature 300°C.

2.5.2. In-source collision induced dissociation (CID) experiments

In-source fragmentation data of the drug and its degradation products were obtained by using in-source CID at collision energy of 35 eV which was found to be suitable to obtain reasonable fragmentation with reproducible signal intensity.

2.5.3. Preparative HPLC method

The degradation product **DP-2** was isolated by using semi preparative HPLC with Water's X-bridge Prep C18 (250 X 10mm, 5µ) (Waters Corporation, Milford, USA) column. The mobile phase composed of 10 mM ammonium acetate (A) and ACN (B) in a gradient method. The gradient elution method was set as follows. (T/ % B): $_{0\text{-}3}$/ 10. $_{3\text{-}5}$/ 35, $_{5\text{-}10}$/ 50, $_{10\text{-}18}$/ 70, $_{18\text{-}21}$/ 80, $_{21\text{-}22}$/ 10, $_{22\text{-}25}$/ 10. The flow rate, injection volume and detection wavelength were 8.0 mL/min, 500µL and 285 nm, respectively. Ethyl acetate was added to the isolated compound. The mixture solution was kept in shaker for 15 min. The supernatant was evaporated on a ScanVac speed Vacuum. The isolated sample was subjected to ^{1}H-NMR study using deuterated MeOH.

2.5.4. Chemical synthesis

The degradation products **DP-6 and DP-7** were synthesized by using lansoprazole as a starting material and *meta*- chloroperoxybenzoic acid (*m*-CPBA) reagent. To a solution of lansoprazole (369 mg, 1.0 mM) in DCM (3ml), m-CPBA (172mg, 1 mM for **DP-6**and 2 mM for **DP-7**) was added at 0^{o} C and the mixture was stirred overnight at room temperature. The reaction mixture was quenched with sodium bicarbonate followed by extraction with ethyl

acetate. After extraction, the residue was purified by using silica gel column chromatography (DCM–MeOH, 9.5:0.5 %) to get the pure products as white solid. The samples were characterized by ^{1}H-NMR using deuterated methanol and a mixture of deuterated methanol and chloroform for **DP-6** and **DP-7,** respectively.

2.6. *In vitro* toxicity evaluation

2.6.1. *In vitro* 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide assay (MTT assay)

The cytotoxic effect of the compounds on normal human cells was determined by performing MTT assay. Briefly, 5 * 10^3 cells of HEK-293 (Human embryonic kidney cells) and RWPE-1(Human prostate epithelial cells) were seeded in each well of 96 well flat bottom tissue culture plates and incubated overnight. Cells were treated with the designated concentration of the degradation products **(DP-2, DP-6 and DP-7)** for 48h; subsequent to the treatment, media was aspirated and 100 µl of MTT (0.5mg/mL) was added and incubated for 4hr and later purple formazan crystals were dissolved in DMSO and absorbance was read at 570nm with a spectrophotometer (Spectramax M4, Molecular devices, USA).

2.7. DNA binding studies

2.7.1. UV-visible spectroscopy

UV-visible absorption spectra were recorded using Perkin Elmer ABI 35 Lambda Spectrophotometer (Waltham, MA, USA) at 25^{o}C. All the experiments were carried out in polystyrene cuvettes to minimize binding of derivatives to the surface of the cuvettes. 50 µM of lansoprazole and degradation products **(DP-2, DP-6 and DP-7)** stock solution was prepared in DMSO and 25.0 µM of ctDNA in 100 mM Tris-HCl (pH 7.0). About 1 mL of 25.0 µM complex solutions was taken in a 1 cm path length cuvette and each time 5µl of DNA was added. Absorption spectra were recorded in the range of 200 nm to 400 nm. All the

solutions used were freshly prepared before commencing the experiment and titration was carried out until saturation of absorbance occurs.

2.7.2. Fluorescence titration

Fluorescence emission spectra were measured at 25^{o}C using a Hitachi F7000 spectrofluormeter (Maryland, USA) using a 1 cm path length quartz cuvette. Quartz cuvettes was thoroughly washed with distilled water and dilute nitric acid (approximately 0.1 N) to minimize non-specific binding of the molecules to the surface of the cuvette. Throughout the fluorescence experiment, the concentration of the lansoprazole and degradation products **(DP-2, DP-6 and DP-7)** were kept constant (10 µM) and titrated with increasing concentrations of ctDNA (multiples of 0.5 µM). Fluorescence spectra were recorded after each addition of ctDNA to the fluorescent cuvette. The complexes were excited at 280nm (lansoprazole), 325 nm (DP-2), 322 nm (DP-6), 278 nm (DP-7) and emission spectra for each titration were recorded from 308 to 365 nm depending upon the molecule. Each spectrum was recorded three times and the average of three scans was taken.

2.7.3. Circular Dichroism studies

DNA conformational studies were carried out on a JASCO 815 circular dichroism spectropolarimeter (Jasco, Tokyo, Japan). Circular dichroism spectroscopic studies were performed to study the change in DNA conformation brought by lansoprazole and degradation products **(DP-2, DP-6 and DP-7)** on the interaction with ctDNA at micro molar concentration range. A solution of ctDNA was prepared in 100mM Tris-HCl (pH 7.0). To 10 µM of ctDNA, about 10.0 µM and 20 µM (1:1 and 1:2 ratios of ctDNA: complex) of each solution containing lansoprazole and degradation products **(DP-2, DP-6 and DP-7)** were

added and CD spectra were recorded from 200 nm to 300 nm in a 1 mm path length cuvette. The spectra were averaged over 3 scans.

2.7.4. Viscosity studies

Viscosity experiments were conducted on Ostwald viscometer, immersed in a water bath maintained at 25^{o}C. Viscosity experiments were performed for lansoprazole and degradation products **(DP-2, DP-6 and DP-7)** (15 µM), after mixing them with ctDNA solution (150 µM). Before mixing DNA and sample, viscosity measurements were performed with ctDNA alone. Ethidium bromide (Et Br)- ctDNA complexes were considered as control. DNA solution was prepared in 100 mM Tris-HCl (pH 7.0). The graph was drawn by plotting $(\eta/\eta o)^{1/3}$ versus complex/ctDNA, where ŋ is the viscosity of ctDNA in the presence of lansoprazole and degradation products and ŋo is the viscosity of ctDNA alone. Viscosity values were calculated according to the protocol mentioned by Tan et al. [21]

3. Results and discussion

3.1. Degradation behaviour of the drug

The degradation behaviour of lansoprazole was analyzed under various stressed degradation conditions. The optimized UPLC/MS method was used to identify the degradation products of lansoprazole. The degradation products of lansoprazole were observed in acid, base, neutral hydrolysis and oxidation. The drug was stable at thermal and photolytic degradation conditions. A total of **8** degradation products, **DP-1** to **DP-8**, were identified and characterized by using UPLC/ESI/HRMS within-source CID experiments. The proposed structures of degradation products of lansoprazole and their elemental compositions are given

in **scheme S1** (**Supplementary Scheme S1, see supporting information) and table 1,** respectively.

3.2.1. Hydrolysis

UPLC/ESI/HRMS analysis of lansoprazole (**Figure 1)** in acid hydrolytic condition showed formation of three degradation products (**DP-1, DP-2 and DP-3**) (**Figure 2(a))** whereas, base (**Figure 2(b))** and neutral (**Figure 2(c))** hydrolysis showed formation of **DP-4** and **DP-5**, respectively.

3.2.2. Oxidation

The drug degraded significantly when subjected to 2% H_2O_2 at room temperature for 30 min. It showed formation of four degradation products (**DP-1**, **DP-6, DP-7 and DP-8**) (**Figure 3(a)**).

3.2.3. Thermal degradation

The exposures of the drug to 80^{o}C for 1week did not show any significant degradation product and it was to be stable under thermal condition **(Figure 3(b))**

3.2.4. Photolytic degradation

Lansoprazole was found to be stable under UV and fluorescence conditions (**Figure 3(c)**).

3.3. In-source CID

3.3.1. Fragmentation of lansoprazole

The positive ion UPLC/ESI/HRMS spectrum of lansoprazole (Rt=20.45 min) shows an abundant $[M+H]^+$ ion at *m/z* 370.In-source CID spectrum shows abundant product ions at *m/z* 136 (loss of HCN from *m/z* 163) and low abundant ions at *m/z* 352. Formation of these ions can be attributed to an ortho effect involving loss of H_2O. Other product ions are at *m/z* 252 (loss of 1*H* –benzo[d]imidazole), *m/z* 234 (loss of $C_7H_4N_2$), *m/z* 222 (loss of $C_2H_3F_3O_2S$), *m/z* 205 (loss of $C_7H_5N_2OS$), *m/z* 204 (loss of $C_7H_6N_2OS$), *m/z* 163(loss of $C_8H_8F_3NO_2$), *m/z* 152(loss of 2,2,2-trifluoro ethanol($C_2H_3F_3O$) from *m/z* 252), *m/z* 122 (loss of $C_7H_4N_2OS$,

$C_2H_3F_3$), *m/z* 119 (loss of $C_9H_8F_3NO_2S$), *m/z* 108 (loss of $C_9H_5F_3N_2O_2S$), *m/z* 107(loss of C_2HF_3O from *m/z* 205), *m/z* 106(loss of C_2HF_3O, $C_7H_6N_2OS$) and *m/z* 122(loss of HCN from *m/z* 119). **(Scheme 1, Figure 4(a)**. While the product ions at *m/z* 252, *m/z* 204, *m/z* 152, *m/z* 122, *m/z* 108 and *m/z* 106 are characteristic of 3-methylpyridine skeleton, *m/z* 119 and 92 are diagnostic of 1H-benzo[d]imidazole skeleton in lansoprazole. The loss of $C_7H_6N_2$and $C_7H_6N_2OS$ from protonated drug clearly indicates that the sulphur monoxide group forms a bridge between benzimidazole and 3-methylpyridine moieties of lansoprazole. The elemental compositions of all these ions have been confirmed by accurate mass measurements (**Supplementary Table S1, see Supporting Information)**.

6.3.2. In-source CID of degradation products

DP-1 ($[M+H]^+$, *m/z* 322)

The degradation product, **DP-1** was formed in acid hydrolysis and oxidation conditions. The elemental composition of its $[M+H]^+$ ions with a mass difference of 48 u compared to that of protonated drug indicates that it is formed by the loss 'SO' from the protonated drug. The in-source CID spectrum of $[M+H]^+$ ion (*m/z* 322) of **DP-1** (Rt = 14.7 min) shows highly abundance product ions at *m/z* 222 (loss of 2,2,2-trifluoroethanol) and low abundance ion at *m/z* 307 (loss of CH_3 radical), *m/z* 239 (loss of $C_2H_2F_3$), *m/z* 238 (loss of 1,1,1-triflouroethane) and *m/z* 224 (loss of C_2HF_3O). These ions clearly indicate the absence of 'SO' group in DP-1 when compared to lansoprazole. **(Supplementary Scheme S2 and Figure 4(b), see Supporting Information)**. Based on these data, **DP-1** was identified as 2-(3-methyl-2-mythylene-4-(2,2,2-trifluoroethoxy) pyridin-1(2*H*)-yl)-1*H*-benzo[d]imidazole. The elemental compositions of all these ions were confirmed by accurate mass measurements (**supplementary Table S2, see Supporting Information**).

DP-2 ($[M+H]^+$, *m/z* 354)

The positive ion UPLC/ESI/HRMS and in-source CID spectrum of $[M+H]^+$ ions (*m/z* 354) of **DP-2 (**Rt = 33.85 min**)**, shows characteristic product ions at *m/z* 321 (loss of SH radical), *m/z* 236 (loss of 1H-benzo[d]imidazole), *m/z* 204 (loss of $C_7H_6N_2S$) and *m/z* 163 (loss of $C_2H_3F_3O$, C_6H_5N from *m/z* 354) for the absence of an oxygen atom in DP-2 as compared to lansoprazole. **(Supplementary Scheme S3 and Figure 4(c), see Supporting Information)**. The elemental compositions of **DP-2** and its product ions have been confirmed by accurate mass measurements (**Supplementary Table S2, see Supporting Information**). Based on these data DP-2 was identified as 2-((3-(4-(2, 2, 2-trifluoroethoxy) pyridin-2-yl) methylthio)-1H-benzo[d]imidazole.

DP-3 ($[M+H]^+$, *m/z* 352)

The in-source CID spectrum of $[M+H]^+$ ions (*m/z* 352) of **DP-3** (Rt = 23.31 min) shows abundant product ions at *m/z* 238 (loss of $C_3H_5F_3O$) and low abundance product ions at m*/z* 306 (loss of CH_2S), at m*/z* 268 (loss of 1,1,1-triflouroethane), *m/z* 252 (loss of $C_2H_3F_3O$), *m/z* 104 (loss of $C_7H_4N_2S$ from *m/z* 252) **(Supplementary Scheme S4 and Figure 4(d), see Supporting Information)**. The mass difference of 18 u between the drug and **DP-3** clearly indicates that it is formed by loss H_2O from the protonated drug. The elemental compositions of **DP-3** and its product ions have been confirmed by accurate mass measurements (**Supplementary Table S3, see Supporting Information**). Based on all these a structure of **DP-3** has been proposed which is shown in **scheme S4**.

DP-4 ($[M+H]^+$, *m/z* 302)

Figure 5(a) shows in-source CID spectrum of $[M+H]^+$ ions (*m/z* 322) of **DP-4** (Rt = 14.6 min) which was formed in base hydrolysis condition. The spectrum shows highly abundant product ions at *m/z* 136 (loss of $C_7H_6N_2OS$, *m/z* 137 (loss of $C_7H_5N_2OS$), *m/z* 122 (loss of $C_8H_8N_2OS$), and low abundance ions at *m/z* 284 (loss of H2O), *m/z* 184 (loss of 1H-benzo[d]imidazole), *m/z* 167 (loss of OH radical from *m/z* 184), *m/z* 166 (loss of H_2O from

m/z 184) and *m/z* 152 (loss of CH_3OH from *m/z* 184) **(Supplementary Scheme S5 and Figure 5(a), see Supporting Information)**. The peaks at *m/z* 284 and 166 are diagnostic ions for the presence of the free –OH group in **DP-4.** The elemental compositions of **DP-4** and its product ions have been confirmed by accurate mass measurements (**Supplementary Table S3, see Supporting Information**). Based on these data, **DP-4** was identified as (Z)-1-(1*H*-benzo[*d*]imidazol-2-yl)-4-methoxy-3-methylpyridin-2(1*H*)-yiidene) methyl) sulfanol.

DP-5 ($[M+H]^+$, *m/z* 272)

The in-source CID spectrum of $[M+H]^+$ ion (*m/z* 272) of **DP-5** (Rt = 11.65 min) shows characteristic highly abundant product ions at *m/z* 119 (loss of C_7H_7NOS), *m/z* 154 (loss of 1H-benzo[d]imidazole) and low abundance ion at *m/z* 239 (loss of SH radical) **(Supplementary Scheme S6 and Figure 5 (b), see Supporting Information)**. The peak at *m/z* 239 is diagnostic ion for the presence of the –S=O group in between benzo imidazole and methylprridine ring of DP-**5**. The elemental compositions of **DP-5** and its product ions have been confirmed by accurate mass measurements (**Supplementary Table S4, see Supporting Information**). Based on these data, **DP-5** was identified as (2-((3-methylpyridine-2-yl) methyl sulfinyl)-1*H*-benzo[*d*]imidazole.

DP-6 ($[M+H]^+$, *m/z* 386)

The degradation product **DP-6** at *m/z*386 formed under oxidation condition. The mass difference between the protonated drug (*m/z* 370) and **DP-6** (*m/z* 386) is 16 Da which suggests the addition of 'O' atom to the degradant**.** The positive ion in-source CID spectrum of $[M+H]^+$ ion of **DP-6 (**Rt = 23.13 min), shows characteristic product ions at *m/z* 119 (loss of $C_9H_8F_3NO_3S$), *m/z* 366 (loss of HF), *m/z* 322 (loss of SO_2), *m/z* 310 (loss of benzyne), *m/z* 268 (loss of $C_7H_4N_2$), and *m/z* 222 (loss of $C_7H_7F_3O$) **(Supplementary Scheme S7 and Figure 5(c), see Supporting Information)**. The loss of SO_2 from **DP-6** yields m/z 322 which is diagnostic for the presence of sulphur dioxide group. In line with this, consecutive

loss of 1H-benzo[d]imidazole and SO_2 clearly indicates that SO_2 forms bridge between benzo imidazole and 3-methylpyridine. The elemental compositions of **DP-6** and its product ions have been confirmed by accurate mass measurements (**Supplementary Table S4, see Supporting Information**). Based on these data, **DP-6** was identified as 2-((3-methyl-4-(2,2,2-trifluoroethoxy) pyridin-2-yl) methyl sulfonyl)-1H-benzo[*d*] imidazole.

DP-7 ([M+H]$^+$, *m/z* 402)

The degradation product, **DP-7** was formed under oxidation condition. The mass difference between the protonated drug (*m/z* 370) and **DP-7** (*m/z* 402) is 32 u which suggests the addition of two oxygen atoms to the degradant**.** The positive ion in-source CID MS/MS spectrum of [M+H] $^+$ions (*m/z* 402) of **DP-7(**Rt = 11.12 min) shows characteristic product ions at *m/z* 322 (loss of SO_3), *m/z* 384 (loss of H_2O), *m/z* 268 (loss of 1H-benzo[d]imidazole and oxygen), *m/z* 222 (loss of $C_7H_4N_2O_2S$), *m/z* 204 (loss of H_2O from *m/z* 222), and *m/z* 119 (loss of $C_8H_8F_3NO_4S$). **(Supplementary Scheme S8 and Figure 5(d), see Supporting Information)**. The peak at *m/z* 322 is a diagnostic ion for the presence of SO_2 group and also indicate that it forms bridge between imidazole ring 2,3-dimethyl-4-(2,2,2-trifluoroethoxy) pyridine moiety. Product ion at *m/z* 384 and *m/z* 119 clearly indicates that oxidation occur at nitrogen of pyridine ring. The elemental compositions of **DP-7** and its product ions have been confirmed by accurate mass measurements (**Supplementary Table S5, see Supporting Information**). Based on these data, **DP-7** was identified as 2-((1H-benzo[d]imidazol-2-ylsulfonyl) methyl)-3-(4-methyl)-4-(2,2,2-trifluoroethoxy) pyridine 1-oxide.

DP-8 ([M+H]$^+$, *m/z* 338)

In-source CID spectrum of [M+H] $^+$ ion of **DP-8** (*m/z* 338) **(**Rt = 27.93 min) shows diagnostic product ions *m/z* 322 (loss of oxygen), *m/z* 254 (loss of 1,1,1-triflouroethane), *m/z* 163(loss of C_6H_5N from *m/z* 254), **(Supplementary Scheme S9 and Figure 5(e), see Supporting Information)** for the presence of ˙O˙ group. The product ion at *m/z* 163 clearly

indicates that oxidation occur at nitrogen of benzo imidazole ring. The elemental compositions of **DP-8** and its product ions have been confirmed by accurate mass measurements (**Supplementary Table S5, see Supporting Information**). Based on these data, **DP-8** was identified as 2-(3-methyl-2-methylene-4-(2, 2, 2-trifluoroethoxy) pyridine-1(2*H*)-yl)-1*H*-benzo[*d*]imidazole 3-oxide.

6.4 Isolation of DP-2

The degradation product **DP-2(Rt=14.2)** was isolated by injecting 500 µL (30 mg/mL) of acidic degradation solution into the semi preparative HPLC. The isolated compound was concentrated for ^{1}H-NMR study (**Supplementary figure S1, see Supporting Information**). The ^{1}H NMR data of **DP-2** was found to be compatible with the proposed structure (**Scheme S3**).

^{1}H NMR (500 MHz, CD_3OD) δ 8.21 – 8.08 (m, 1H), 7.48 – 7.26 (m, 2H), 7.15 – 7.07 (m, 2H), 6.98 – 6.82 (m, 1H), 4.59 (dd, *J* = 16.5, 8.3 Hz, 3H), 4.53 (s, 2H), 2.17 (s, 3H).

7. Procedure for synthesis of DP-6 and DP-7

To synthesize the **DP-6 and DP-7,** 1 mM and 2 mM *m*-CPBA (172mg) was added separately to each of the two solutions of lansoprazole (369 mg, 1 mM) in 3mL of DCM, respectively. The reaction mixture was stirred at room temperature for overnight. After the reaction was completed, the reaction mixture was quenched with $NaHCO_3$ followed by extraction with ethyl acetate (15 mL) and the organic layer was separated. The organic layer was dried over on anhydrous magnesium sulfate and concentrated under vacuum to get dry product. The residue was purified by silica gel column chromatography (DCM–MeOH, 9.5:0.5 %) to afford pure products **DP-6** and **DP-7** as a white solid. Pure solid compounds were subjected to ^{1}H NMR experiment study using deuterated solvents CD_3OD and $CDCl_3$ + CD_3OD mixture for **DP-6** and **DP-7,** respectively. The ^{1}H NMR data confirmed the **DP-6** and **DP-7**

(**Supplementary figure S2, figure S3, Scheme S7 and Scheme S8, see Supporting Information)**.

DP-6: ^{1}H NMR (400 MHz, CD_3OD) δ 7.94 (d, *J* = 5.7 Hz, 1H), 7.58 (s, 2H), 7.36 – 7.29 (m, 2H), 6.89 (d, *J* = 5.7 Hz, 1H), 4.95 – 4.78 (m, 2H), 4.58 (q, *J* = 8.3 Hz, 2H), 2.17 (s, 3H).

DP-7: ^{1}H NMR (300 MHz, CD_3OD+$CDCl_3$) δ 7.87-7.94 (m, 1H), 7.55-7.64 (m, 2H), 7.26 – 7.36 (m, 2H), 6.97-6.99 (d, *J* = 7.3 Hz, 1H), 4.50 – 4.59 (m, 2H), 3.26 (s, 2H), 2.30 (s, 3H).

7. Mechanism for the formation of degradation products

7.1. Hydrolytic Degradation products

Formation of **DP-1 and DP-3** can be explained by abstraction of an acidic proton by 'N' of the imidazole ring followed by nucleophilic attack of pyridine and cleavage of C-S bond to form an intermediate with free S-OH group. The consecutive losses of 'SO' and H_2O molecules can lead to the formation of **DP-1 and DP-3**, respectively (**Scheme 2**). Formation of **DP-2** can be explained by removal of oxygen from the drug which may involve a radical cation mechanism [21] (**Scheme 2**). **DP-4** can be formed by a mechanism involving a nucleophilic attack by hydroxide anion on trifluoro carbon followed by loss of CF_3OH (**Scheme 2).** Formation of **DP-5** can be explained by hydrolysis, leading to O-CH_2CF_3 bond cleavage followed by loss of C_2HF_3O (**Scheme 3**).

7.2. Oxidative Degradation products

The oxidative degradation product **DP-6** can be explained by a nucleophilic attack of peroxide on sulphur of the drug followed by H_2O loss to form **DP-6.** The intermolecular nucleophilic attack of 'N' of pyridine on imidazole carbon of **DP-6** and cleavage of C-S bond to form an intermediate with the free SO_2H group followed by loss H_2SO_2 to form **DP-8**. The **DP-7** is the N-oxide of **DP-6 (Scheme 3)**.

8. MTT assay

Degradation products **DP-2, DP-6** and **DP-7** were evaluated for toxicity on HEK-293 and RWPE-1 cell lines. Lansoprazole has exerted 3.1% loss of viability at 100 µm concentration whereas **DP-2, DP-6** and **DP-7** have displayed 20.8, 46.5, 12 % loss of viability in RWPE1 cell line. Similarly, lansoprazole has exerted 8.5% loss of viability at 100 µm concentration. **DP-2 DP-6** and **DP-7** has displayed 19.7, 39.1, 12 % loss of viability in HEK293 cells (**Figure 6**).

9. DNA binding studies

Lansoprazole, **DP-2**, **DP-6** and **DP-7** have shown maximum absorption at around 272, 283 and 285 nm. On addition of equal aliquots of ctDNA, the ligand absorption peaks exhibited hyperchromicity accompanied by a slight blue shift. It is known that hypochromicity along with bathochromic shift (red shift) is considered as an evidence of intercalative mode of binding. The extent of hypochromicity of the absorption band can be taken as a measure of intercalative binding strength. [21] The absorption band hypochromicity is usually attributed to the interaction between the electronic states of the complex and the DNA bases. [22] From UV-visible titration data, the LAN molecules ctDNA binding constants were found to be in the range of $\approx 2 \times 10^4$ M^{-1}. The hyperchromic effect may also be due to the electrostatic interaction between lansoprazole, **DP-2**, **DP-6**, **DP-7** molecules and the phosphates in the backbone at the periphery of the double helix ctDNA. [23] Hyperchromisity of ligand absorption bands on addition of ctDNA, indicate that the lansoprazole, DP-2, DP-6, DP-7 molecules interact with DNA and they may bind electrostatically to the surface of ctDNA. But only with UV-Visible titration data alone, is not possible to understand the mode of complex molecules binding to DNA. The representative UV-visible spectra obtained on interaction of complex with ctDNA were shown in **Figure 7 (a).**

Fluorescence titration is yet another valuable technique for understanding the binding mode of small molecules with DNA and to study the electronic environment around the DNA-complex at comparatively lower concentrations. [24] Since the complexes are fluorescent, their interaction with DNA can be monitored at low concentration. Lansoprazole, **DP-2**, **DP-6** and **DP-7** emission spectra shows a prominent peak in the range from 308 nm to 368 nm. On addition of equal increments of ctDNA to the solution of the drug and **DP-2**, **DP-6** and **DP-7**, the fluorescence emission intensity has increased gradually, which is consistent with the non-intercalative mode of binding such as electrostatic binding mode (surface binding) that leads to effective protection of the drug, **DP-2**, **DP-6** and **DP-7** by DNA. The hydrophobic environment inside the DNA helix reduces the drug, **DP-2**, **DP-6** and **DP-7** accessibility to the solvent molecules. As a result, the complex mobility gets restricted at binding site, leading to a decrease of the vibrational modes of relaxation. This will result in enhancement of emission intensity or higher emission intensity. [25] The representative fluorescence spectra are shown in **Figure 7(b).**

In order to understand the effect of the drug, **DP-2**, **DP-6** and **DP-7** on ctDNA conformation, circular dichroism studies were performed. The CD spectra of ctDNA exhibited positive and negative bands at 275 nm and 245 nm respectively. At an average all the drug, **DP-2**, **DP-6** and **DP-7**demonstrated around 10-12 % hypochromicity of positive CD band on interaction with CT DNA. On addition of 10 μM the drug, **DP-2**, **DP-6** and **DP-7** molecules to ctDNA (at 1:1 ratio), the positive CD band showed hypochromicity, indicating unwinding of ctDNA on interaction with them [25]. Further, on doubling the concentration (at 1:2 ratio), the positive band intensity increased slightly. Hyperchromicity of the positive CD band is an indication of stabilization of the DNA-ligand complex upon further interaction between the bound drug, **DP-2**, **DP-6** and **DP-7.** [26] CD studies indicate that the drug, **DP-2**, **DP-6** and **DP-7** interact with DNA and bring small changes in DNA conformation. It was found that,

the extent of change in DNA conformation is more with **DP-7** compared to other molecules. The negative CD band intensities reduced in case of all the complexes, but maximum change was noticed with **DP-7**, emphasizing the role of these molecules in effecting the DNA helix. The CD spectra obtained with the drug, **DP-2**, **DP-6** and **DP-7** and ctDNA was shown in **Figure 7(c).**

The spectroscopic studies indicate that the drug, **DP-2**, **DP-6** and **DP-7** interact well with DNA. In order to understand the nature of interaction of the drug, **DP-2**, **DP-6** and **DP-7** with double helical ctDNA, viscosity studies were carried out. Relative viscosity of DNA increases when the molecules intercalate with DNA. The enhancement of viscosity is due to increase in the axial length of DNA helix on molecule's intercalation. Hence, the intercalation of a compound results in an increase of the viscosity of DNA solutions [27] whereas, the reduction in the relative viscosity is typically observed with covalent DNA binding. [28] Viscosity does not change or show very less change when molecules bind to the surface of DNA. [29]

On complex intercalation, the axial length of DNA helix must increase to accommodate the binding complex molecules, which leads to the increase of DNA solution viscosity. Itis noticed that the viscosity of DNA solution increased slightly on addition of the drug, , **DP-2**, **DP-6** and **DP-7**.Minor positive or negative changes in DNA solution viscosity are observed when binding occurs in the DNA grooves [29] As shown in **Figure 7 (d),** on the interaction of the drug, **DP-2**, **DP-6** and **DP-7** molecules with DNA exhibited a slight increase in the viscosity, which is not as pronounced as observed for classical intercalators [30] and are consistent with substrates that bind to ctDNA through a groove-binding mode.[31]Among all the DPs, **DP-2**, **DP-6** and **DP-7**, change in viscosity was more in case of **DP-7** whereas, with the others DPs, change DNA viscosity was comparatively low, may due to lesser interaction

with ctDNA. But on the other hand, when 15 μM of Et Br was added to ctDNA, the viscosity of DNA has increased continuously. This is due to intercalation of Et Br molecules with ctDNA. The graph plotted between $(\eta/\eta_o)^{1/3}$ and complex/ctDNA was shown in **Figure 7(d).** Spectroscopic and viscosity studies indicate that the the drug, **DP-2**, **DP-6** and **DP-7** moleculesinteract well with DNA and they exhibit groove binding mode of interaction with DNA.

8. Conclusion

Forced degradation studies of lansoprazole, carried out as per ICH guide lines, gave rise to a total of 8 degradation products. Five of these DPs are hitherto unknown. The major degradation products, DP-2 was isolated by semi preparative HPLC and DP-6 and DP-7 were synthesized and characterized by 1H-NMR. The cytotoxic effect of degradation products was tested on normal human cells such as HEK 293 (embryonic kidney cells) and RWPE-1 (normal prostate epithelial cells) by MTT assay. From the results of cytotoxicity, it was found that lansoprazole as well as its degradation products (DP-2, DP-6 and DP-7) were nontoxic up to 50 μM concentration and overall it was found that the degradation products showed slightly higher cytotoxicity when compared with that of the cytotoxicity of lansoprazole. In order to understand the nature of binding and interaction of DP-2, DP-6 and DP-7 molecules with double helical ctDNA, spectroscopic and viscosity studies were performed. These studies indicate that DP-2, DP-6 and DP-7 molecules interact with ctDNA and they may bind to its surface.

Acknowledgement

The authors thank Dr. S Chandrasekhar, Director, IICT, Hyderabad for facilities and their cooperation. Financial support was provided partially by CMET (CSC0110) and AARF (CSC0406) projects. G.S is thankful to U.G.C, New Delhi, for awarding Junior Research

Fellowship. R.M.B and S. U are thankful to CSIR, New Delhi, for awarding Senior Research Fellowship and Junior Research Fellowship, respectively.

References

1. ICH. Stability testing of new drug substances and products Q1A(R2). *International Conference on Harmonization*, *IFPMA*, Geneva, Switzerland, **2000**.
2. B. Raju, M. Ramesh, R. Srinivas, S.S. Raju, Y. Venkateswarlu. Identification and characterization of stressed degradation products of prulifloxacin using LC-ESI-MS/Q-TOF, MS^n experiments: development of a validated specific stability-indicating LC-MS method. J. *Pharm. Biomed. Anal*.**2011**, 56, 560.
3. R.M. Borkar, B. Raju, R. Srinivas, S.K. Shetty. Identification and characterization of stressed degradation products of metoprolol using LC/Q-TOF-ESI-MS/MS and MS(n) experiments. *Biomed Chromatogr*.**2012**,26, 720.
4. R.M. Borkar, B. Raju, P.S. Devrukhakar, N.Tondepu, A.B.N. Rao. R. Srinivas. Liquid chromatography/electrospray ionization tandem mass spectrometric study of milnacipran and its stressed degradation products. *Rapid Commun. Mass Spectrom.***2013**, 27, 369.
5. Betto, L. Turchetto, L. Longinotti. Allergenic impurities in medicinal preparations containing aminopenicillin. I. Ampicillin sodium. *AnnaliDell'IstitutoSuperiore di Sanità*. **1989**, 25, 315.
6. N. Entwistle, P. Owen, D. Patterson, L. Jones, J. Smith. The occurrence of chlordiazepoxide degradation products in sudden deaths associated with chlordiazepoxide over dosage. *J Forensic Sci Soc*.**1986**, 26, 45.
7. M. M. Bhandi, R. M. Borkar, G. Shankar, S.Raut, N. Nagesh and R. Srinivas. Identification and characterization of stressed degradation products of rabeprazole using LC/ESI/MS/MS and 1H-NMR experiments: In vitro toxicity evaluation of major degradation products. *RSC Adv*.**2016**, 6, 10719.
8. ICH. Impurities in new drug products Q3B (R2). *International Conference on Harmonisation, IFPMA*, Geneva (Switzerland), **2006**.
9. ICH. Impurities in new drug substances Q3A (R2). *International Conference on Harmonisation. IFPMA*, Geneva (Switzerland), **2006**.

10. ICH guideline. Q1B Photostability Testing on New Drug Substances and Products. International *Conference on Harmonization*, *IFPMA*, Geneva, Switzerland, **2000**.
11. ICH. Q5C Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products, Geneva, Switzerland, **1995**.
12. WHO. Expert Committee on Specification for Pharmaceutical Preparations, WHO Technical Report Series 953, Annex 2, Stability Testing of Active Pharmaceutical Ingredients and Finished Pharmaceutical Products, Geneva, Switzerland, **2009**.
13. USFDA. Guidance for Industry: Stability Testing of Drug Substances and Drug Products (Draft Guidance), Rockville, USA, **1998**http://www.fda.gov/ohrms/dockets/98fr/980362gd.pdf.
14. EMEA, CPMP. Guideline on Stability Testing: Stability Testing of Existing Active Substances and Related Finished Products, London, UK, **2003**.
15. M.Noubarani, F. Keyhanfar, M. Motevalian, M. Mahmoudian.Improved HPLC method for determination of four PPIs, omeprazole, pantoprazole, lansoprazole and rabeprazole in human plasma. *J Pharm Pharm Sci*.**2010**, 13, 1.
16. H. Wang, Y. Sun, X.Meng, B. Yang, J. Wang, Y. Yang, J.Gu. Determination of lansoprazole enantiomers in dog plasma by column-switching liquid chromatography with tandem mass spectrometry and its application to a preclinical pharmacokinetic study. *J Sep Sci*.**2015**, 17, 2960.
17. R.Cirilli, R. Ferretti, B.Gallinella, L.Turchetto, L.Zanitti, F. La Torre. Development and validation of an enantioselective and chemoselective HPLC method using a Chiralpak IA column to simultaneously quantify (R)-(+)- and (S)-(-)-lansoprazole enantiomers and related impurities. *J Pharm Biomed Anal*.**2009**, 15, 9.
18. K.Ramulu, B.M. Rao, N.Someswara Rao. Identification, Isolation and characterization of potential degradation product in lansoprazole drug substance.*Rasayanjournal*.**2013**, 6, 274.
19. B.Satyanarayana, P. Vasudev. Hydrolytic Degradation Study of Lansoprazole, Identification, Isolation and Characterisation of Base Degradation Product. *American Journal of Analytical Chemistry*.**2015**, 6, 145.

20. C.P. Tan, J. Liu, L.M. Chen, S. Shi, L.N. Ji. Synthesis, structural characteristics, DNA binding properties and cytotoxicity studies of a series of Ru(III) complexes. *J. Inorg. Biochem.***2008**, 102, 1644.

21. J.K. Barton, J. J. Dennenberg, J.B.Chaires. Tris(phenanthroline)ruthenium(II) enantiomer interactions with DNA: Mode and specificity of binding. *Biochemistry*.**1993**, 32,2573.

22. B. D. Wang, Z. Y. Yang, P. Crewdson, D. Q. Wang. Synthesis, crystal structure and DNA-binding studies of the Ln(III) complex with 6-hydroxychromone-3-carbaldehyde benzoyl hydrazone. *J Inorg Biochem*. **2007**, 101,1492.

23. E.C. Long and J.K. Barton. On demonstrating DNA intercalation. *Acc Chem Res.***1990**,23, 271.

24. J.Wu, M.Zhao, K. Qian, K.H Lee,S. Morris-Natschke, S. Peng.Novel N-(3-carboxyl-9-benzyl-beta-carboline-1-yl)ethylamino acids: synthesis, anti-tumor evaluation, intercalating determination, 3D QSAR analysis and docking investigation.*Eur J Med Chem*.**2009**, 44, 4153.

25. H.L.Chan, D.L.Ma, M.Yang, C.M.Che.Bis-intercalative dinuclear platinum (II) 6-phenyl-2, 2′-bipyridine complexes exhibit enhanced DNA affinity but similar cytotoxicity compared to the mononuclear unit.*J Biol Inorg Chem*. **2003**, 8,761.

26. H. Fukuda, M. Katahira, N. Tsuchiya, Y. Enokizono, T. Sugimura, M. Nagao, H. Nakagama. Unfolding of quadruplex structure in the G-rich strand of the minisatellite repeat by the binding protein UP1.*PNAS*.**2002**, 99,12685.

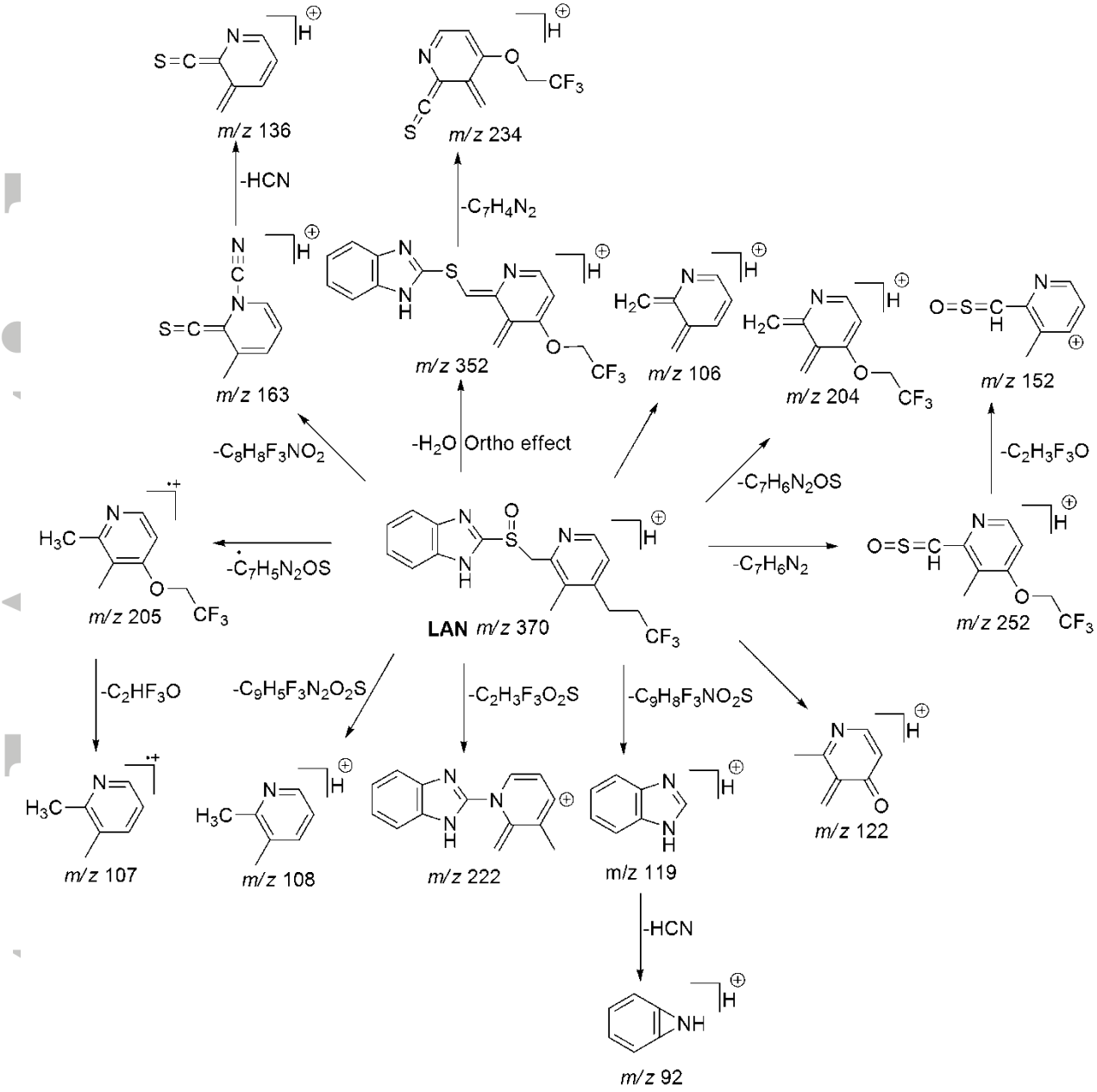
27. L.Wang, Y.Wen, J. Liu, J. Zhou, C. Li, C.Wei. Promoting the formation and stabilization of human telomeric G-quadruplex DNA, inhibition of telomerase and cytotoxicity by phenanthroline derivatives. *Org Biomol Chem.***2011**, 9, 2648.

28. N. Shahabadi, S. Kashanian, and M. Purfoulad. DNA interaction studies of a platinum(II) complex, PtCl(2) (NN) (NN=4,7-dimethyl-1,10-phenanthroline), using different instrumental methods. *Spectro chimica Acta A*, **2009**, 72,757–761.

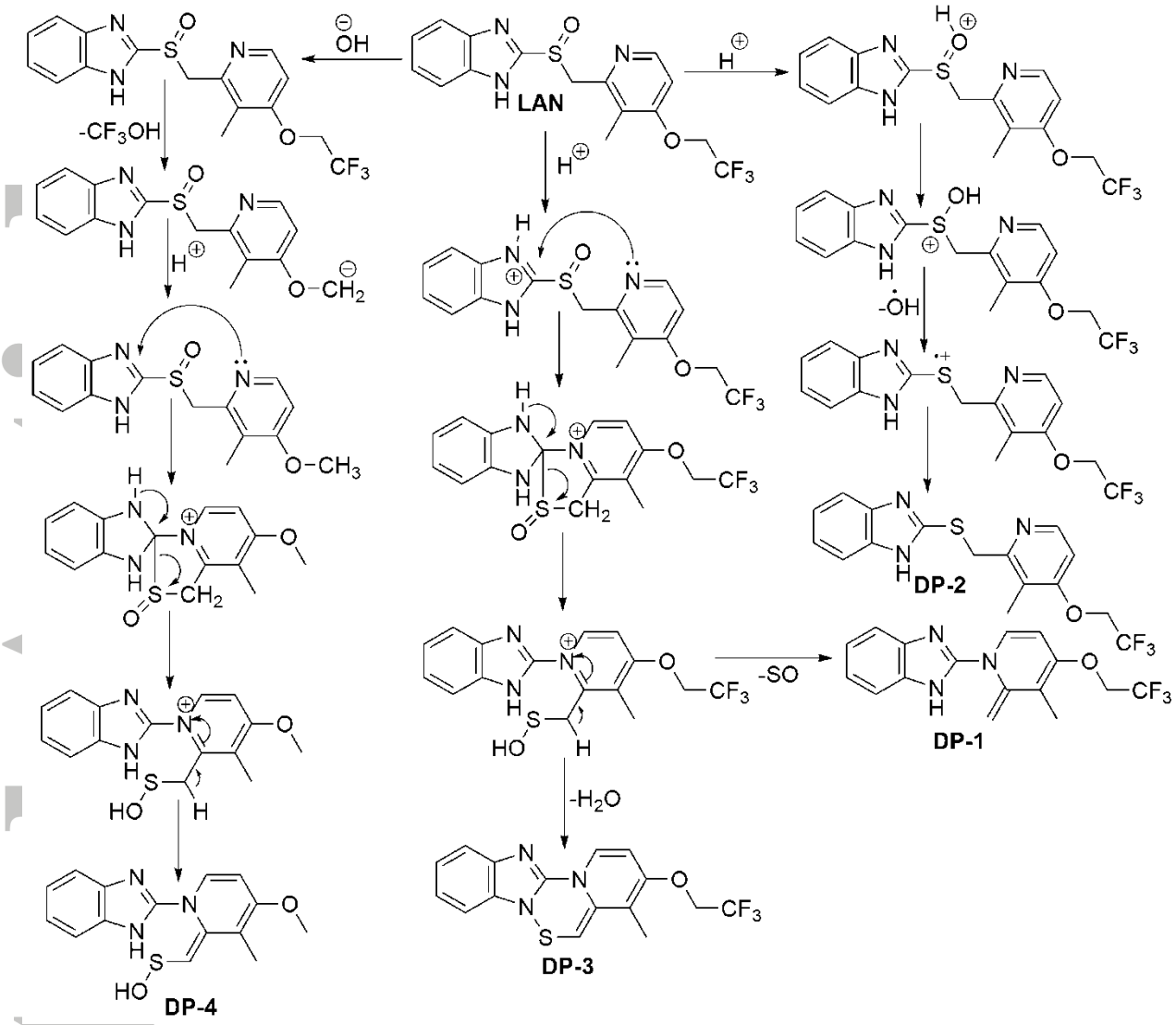
29. J. M. Kelly, A. B. Tossi, D. J. Mcconnell, and C. Ohui-gin.A study of the interactions of some polypyridylruthenium (II) complexes with DNA using fluorescence spectroscopy, topoisomerisation and thermal denaturation. *Nucleic Acids Research.***1985**, 13,6017.

30. V. G. Vaidyanathan and B. U. Nair.Photooxidation of DNA by a cobalt(II) tridentate complex. *J. Inorg. Biochem*.**2003**, 94, 121.

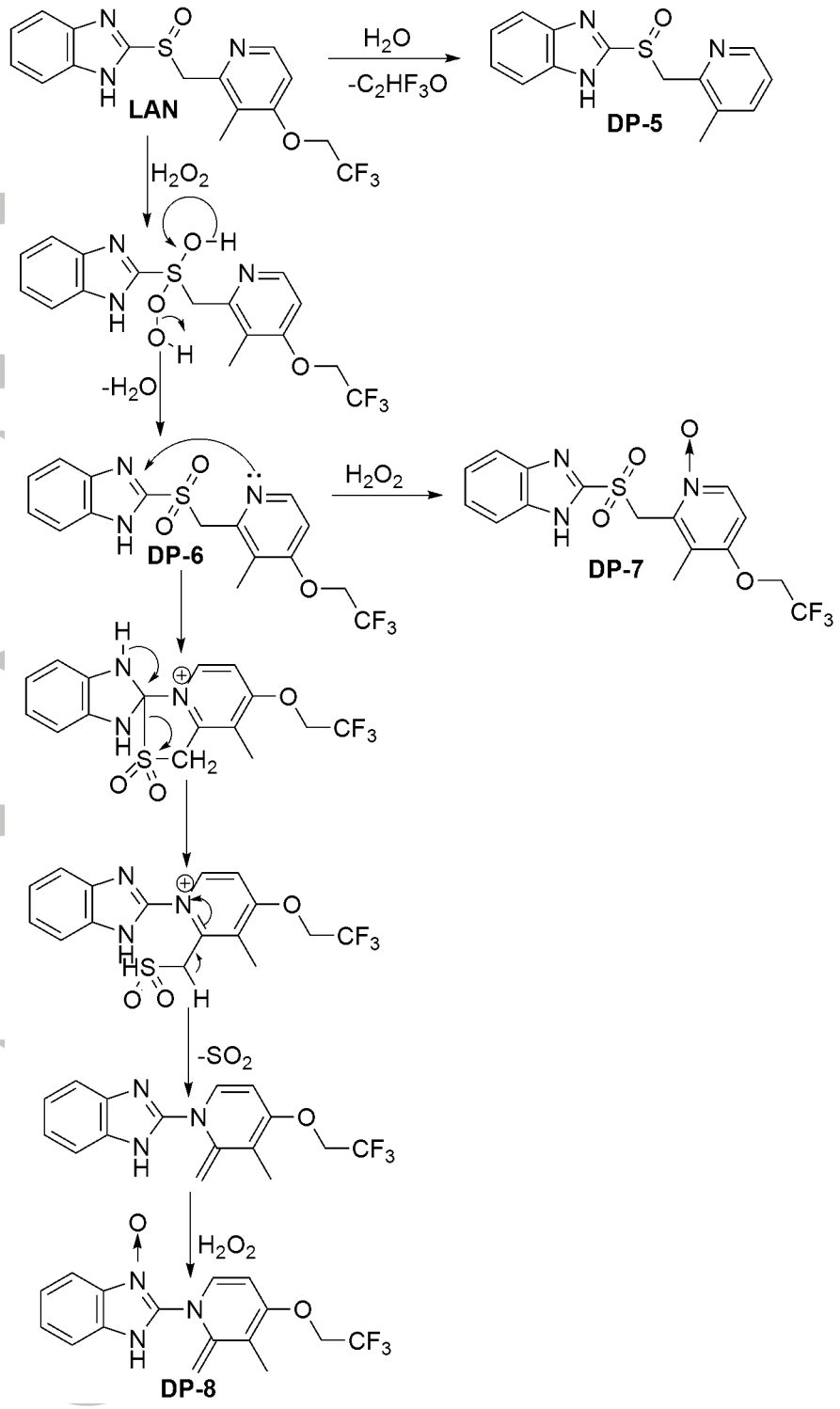
31. C. Metcalfe, C. Rajput and J. A. Thomas. Studies on the interaction of extended terpyridyl and triazine metal complexes with DNA. *J. Inorg. Biochem.***2006**, 100, 1314.



Scheme 1: Proposed fragmentation pathway of the protonated drug (***m/z* 370**)



Scheme 2: Probable mechanism for the formation of degradation products (**DP-1, DP-2, DP-3, and DP-4**) under acid and base hydrolysis conditions



Scheme 3: Probable mechanism for the formation of degradation products (**DP-5, DP-6, DP-7 and DP-8**) under neutral hydrolysis and oxidation conditions

Table 1: UPLC/ESI/HRMS data of the drug and its degradation products

Degradation products	Retention time(min)	Proposed molecular formula $[M+H]^+$	Observed(*m/z*)	Calculated (*m/z*)	Error (ppm)
LAN	20.45	$C_{16}H_{15}F_3N_3O_2S^+$	370.08320	370.08316	-0.10
DP-1	14.41	$C_{16}H_{15}F_3N_3O^+$	322.11568	322.11617	1.52
DP-2	33.85	$C_{16}H_{15}F_3N_3OS^+$	354.08791	354.08824	0.93
DP-3	33.14	$C_{16}H_{13}F_3N_3OS^+$	352.07328	352.07259	-1.95
DP-4	14.54	$C_{15}H_{16}N_3O_2S^+$	302.09642	302.09577	-2.10
DP-5	11.65	$C_{14}H_{14}N_3OS^+$	272.08487	272.08521	1.24
DP-6	11.05	$C_{16}H_{15}F_3N_3O_3S^+$	386.07834	386.07807	0.69
DP-7	23.13	$C_{16}H_{15}F_3N_3O_4S^+$	402.07002	402.07299	7.38
DP-8	27.94	$C_{16}H_{15}F_3N_3O_2^+$	338.11234	338.11109	-3.69

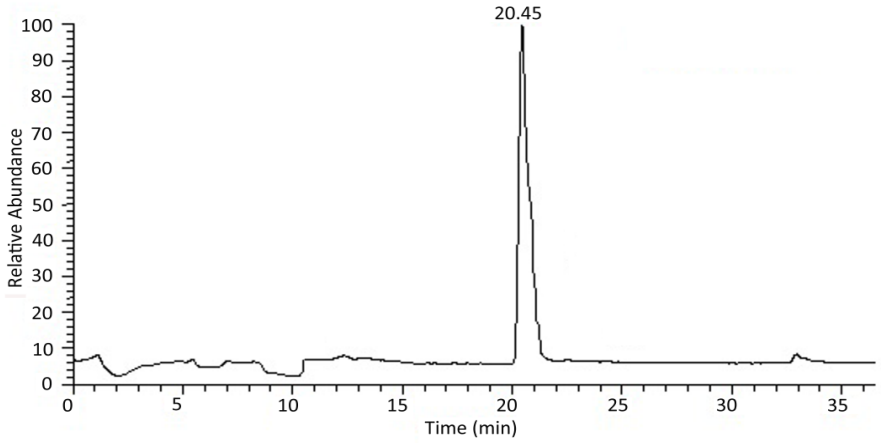


Figure 1. UPLC-ESI-MS TICs of lansoprazole

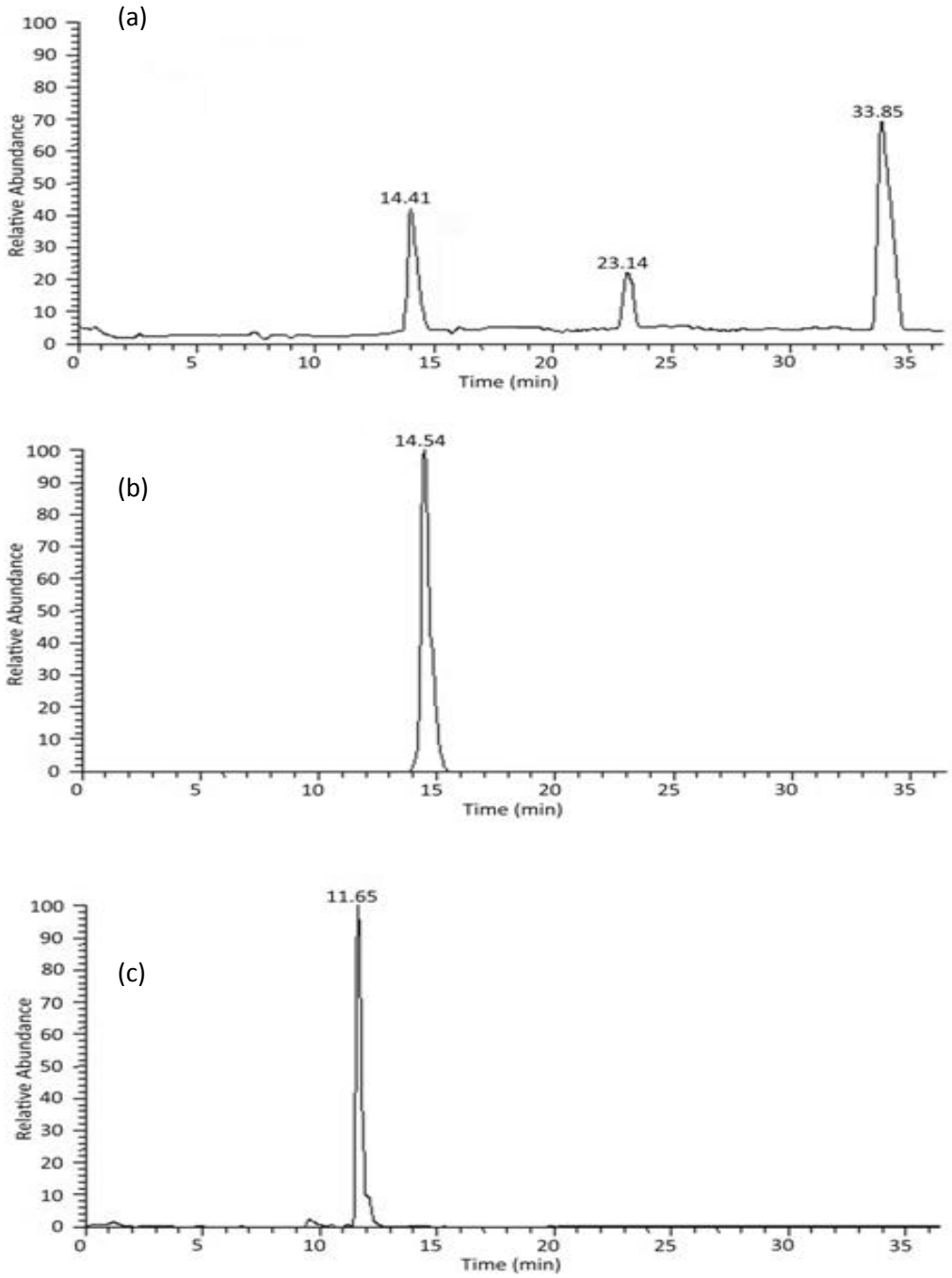


Figure 2. UPLC/ESI/MS TICs of lansoprazole degradation products under acid (a), base (b), and neutral (c) hydrolytic conditions.

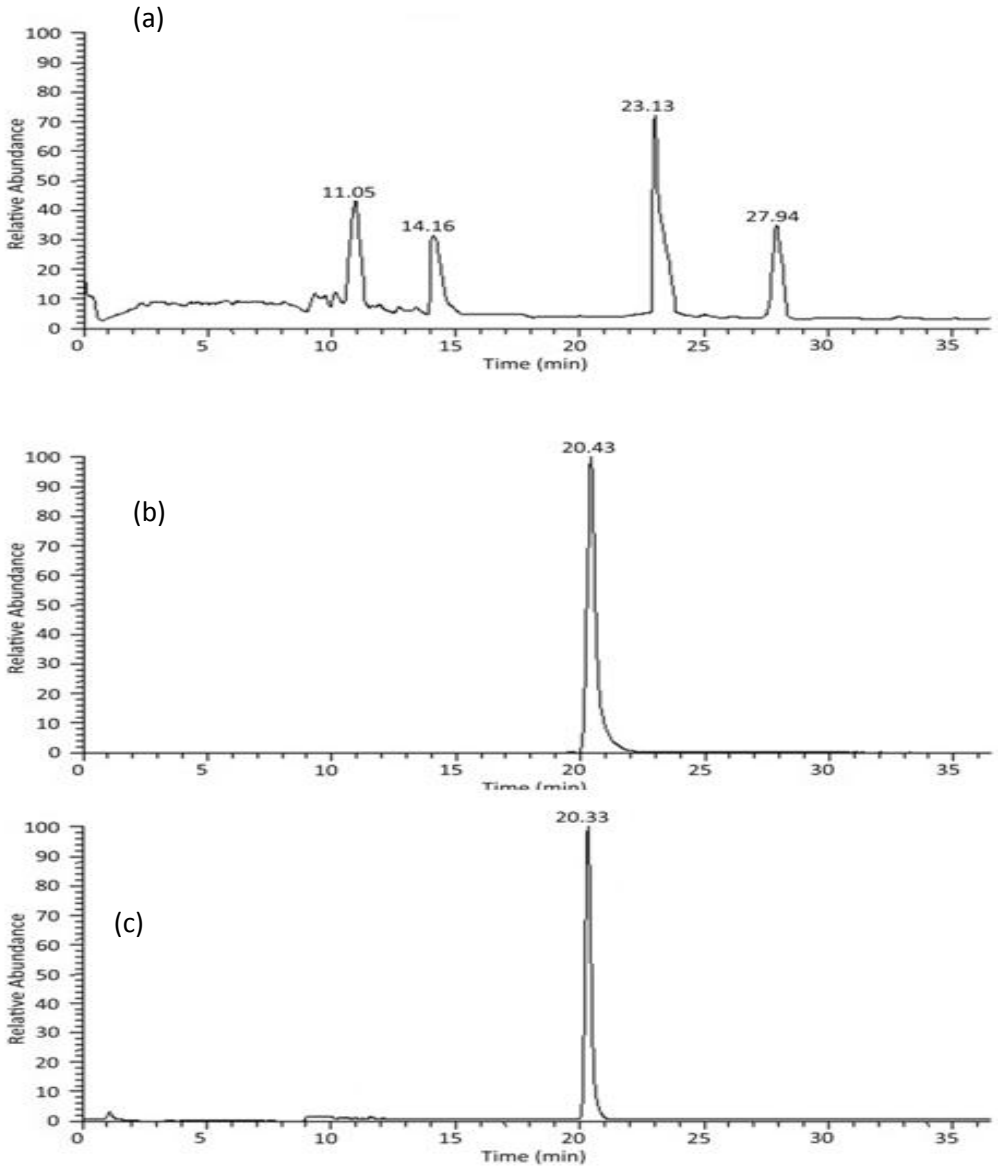


Figure 3. UPLC/ESI/MS TICs of lansoprazole degradation products under oxidation (a), thermal (b) and photolytic (c) degradation conditions.

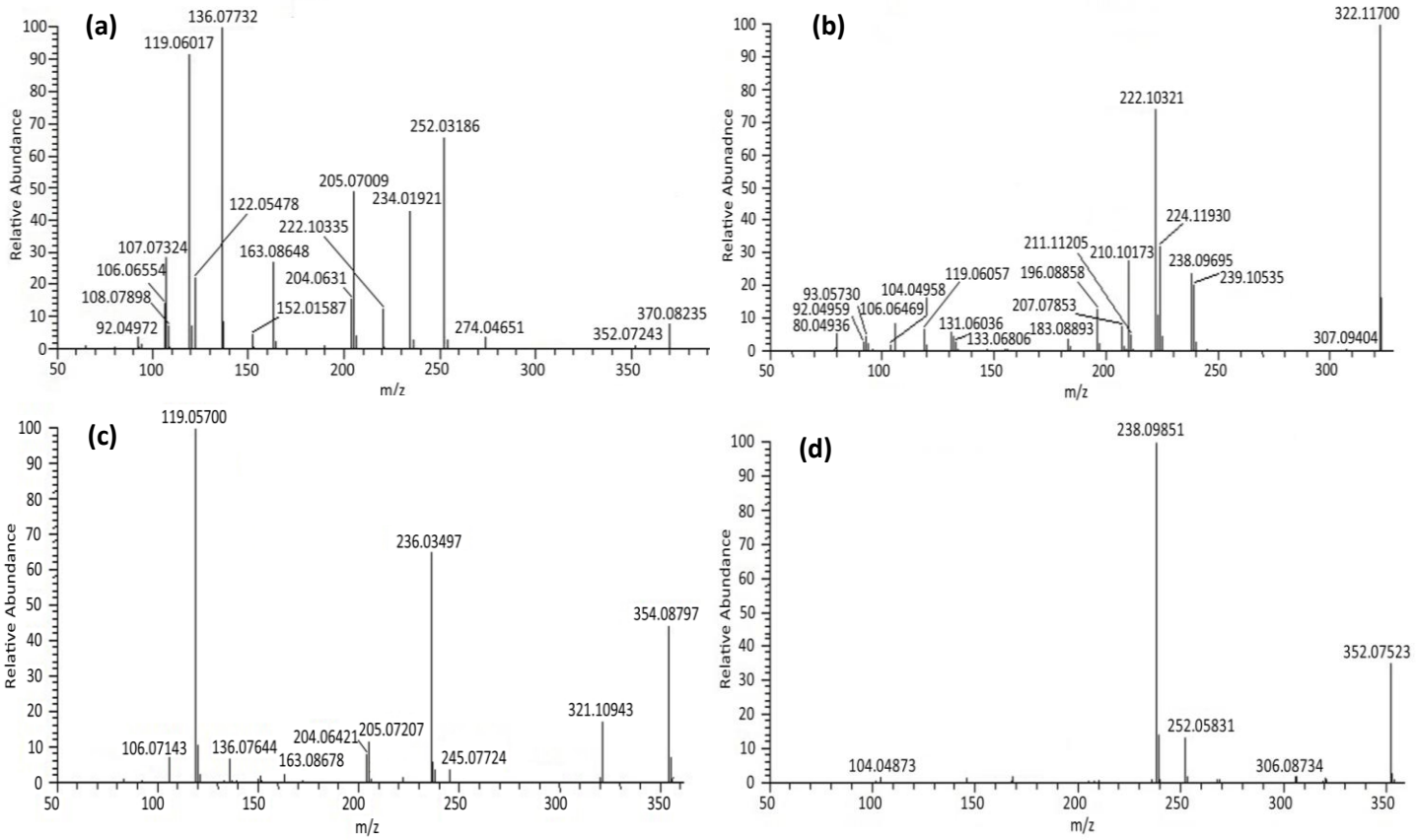


Figure 4. UPLC/ESI/in-source CID MS Spectrum of $[M+H]^+$ ion of **(a)** Lansoprazole (*m/z* 370.08235), **(b) DP-1** (*m/z* 322.11700), **(c) DP-2** (*m/z* 354.08797), **(d) DP-3** (*m/z* 352.07523) at 35 eV.

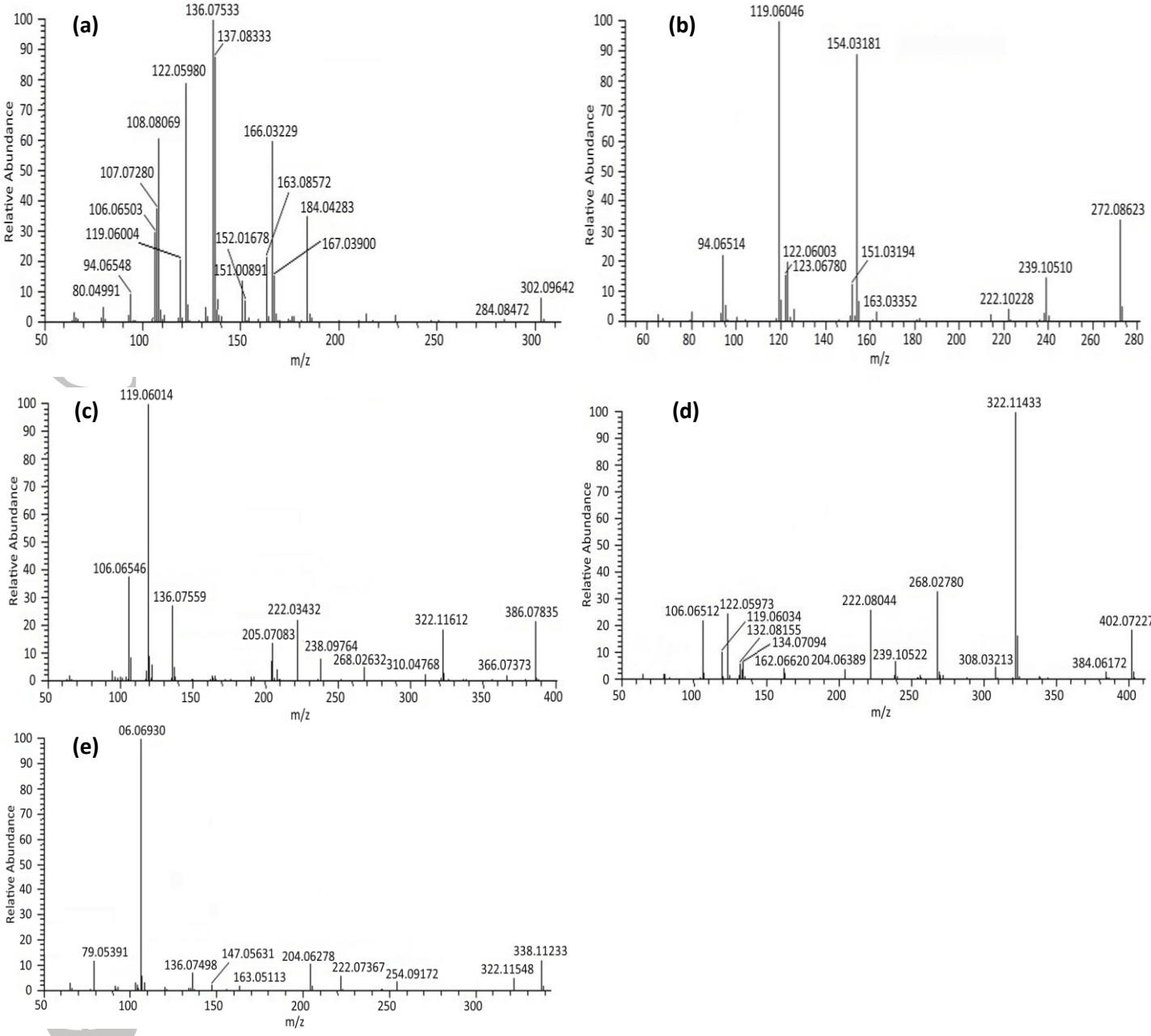


Figure 5. UPLC/ESI/in-source CID MS Spectrum of $[M+H]^+$ ion of **(a) DP-4** (*m/z* 302.09642), **(b) DP-5** (*m/z* 272.08623), **(c**) **DP-6** (*m/z* 386.07835), **(d) DP-7** (*m/z* 402.07227), **(e) DP-8** (*m/z* 338.11233) at 35 eV.

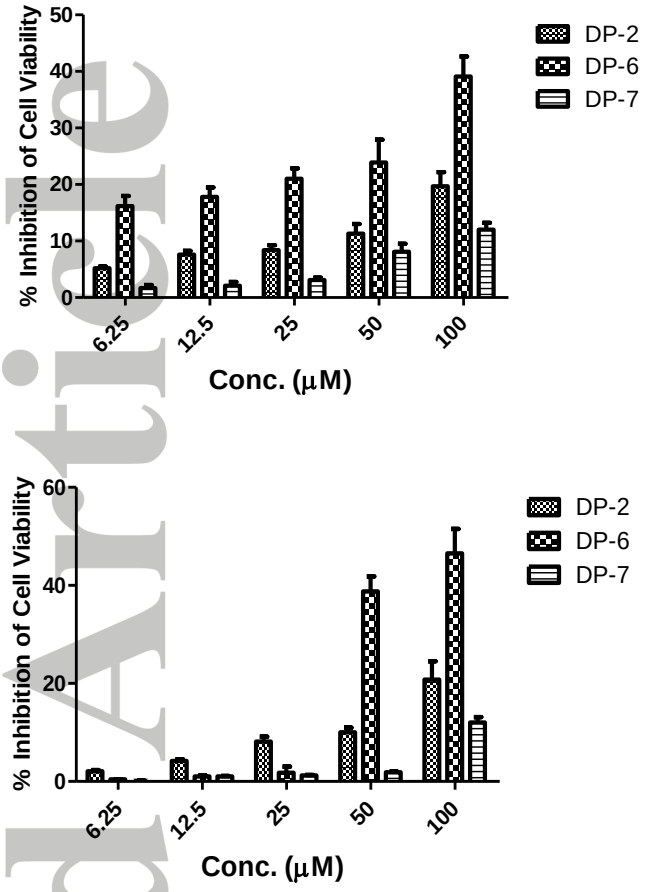


Figure 6. Graphical representation of the inhibitory effect of degradation products **DP-2, DP-6** and **DP-7** on cell viability of (a) HEK293 and (b) RWPE-1 cell lines

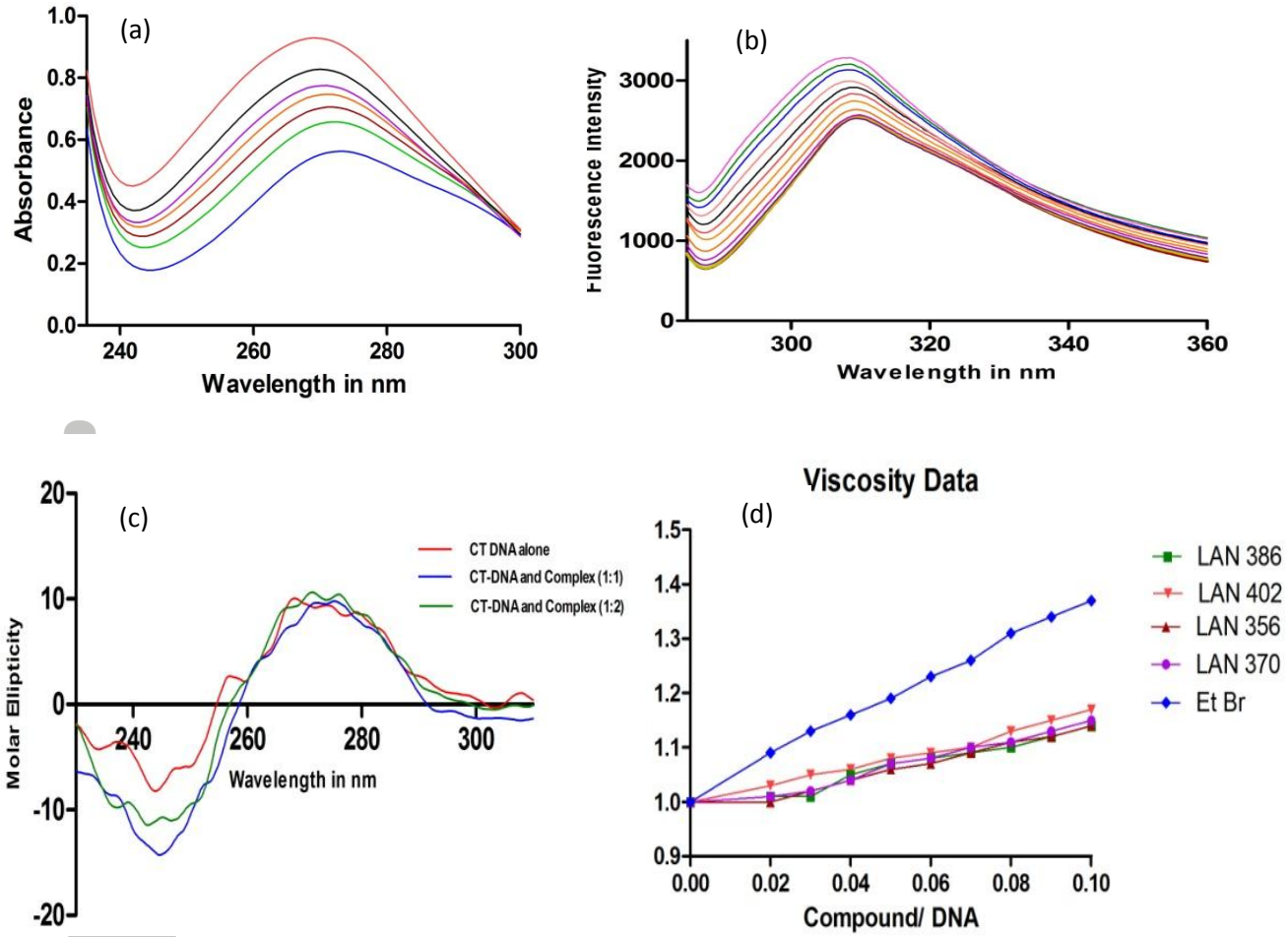


Figure 7. A representative figure of **(a)** UV-Visible spectra (b) Fluorescence emission spectra (c) Circular dichorism spectra and (d) Viscosity spectra of RT complex on interaction with ctDNA.