Chapter 3

Identification and characterization of a principal oxidation impurity in Clopidogrel drug substance and drug product
3.1 INTRODUCTION

Clopidogrel bisulfate, methyl (+)-(S)-α-(2-chlorophenyl)-6,7-dihydrothieno-[3,2-c]pyridine-5(4H)-acetate sulfate (1:1) (Fig. 3.1), is an oral, thienopyridine class antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease. It is marketed by Bristol-Myers Squibb and Sanofi-Aventis under the trade name Plavix, marketed worldwide in nearly 110 countries, with sales of US$6.6 billion in 2009[1]. It had been the second top selling drug in the world for few years as of 2007[2] and was still growing by over 20% in 2007. U.S. sales were US$3.8 billion in 2008.[3] It is also marketed by various leading pharmaceuticals manufacturers as shown in the table 3.1. Clopidogrel is a prodrug and the mechanism is irreversible blockade of the adenosine diphosphate (ADP) receptor P2Y12 and is important in platelet aggregation, the cross-linking of platelets by fibrin. The blockade of this receptor inhibits platelet aggregation by blocking activation of the glycoprotein IIb/IIIa pathway. Platelet inhibition can be demonstrated 2 hours after a single dose of oral clopidogrel, but the onset of action is slow, so that a loading-dose of 300–600 mg is usually administered.[4]

Clopidogrel is rapidly absorbed after oral administration of repeated doses of 75 mg clopidogrel (base), with peak plasma levels (appx. 3 mg/l) of the main circulating metabolite occurring approximately one hour after dosing. The pharmacokinetics of the main circulating metabolite is linear (plasma concentrations increased in proportion to dose) in the dose range of 50 to 150 mg of clopidogrel. Absorption is at least 50% based on urinary excretion of clopidogrel-related metabolites. Clopidogrel and the main circulating metabolite bind reversibly in vitro to human plasma
proteins (98% and 94%, respectively). The binding is nonsaturable \textit{in vitro} up to a concentration of 110 g / ml.

3.2 DRUG PROFILE

Clopidogrel is a white to off-white powder and it is practically insoluble in water at neutral pH but freely soluble at pH 1. It also dissolves freely in methanol, dissolves sparingly in methylene chloride. It has a specific optical rotation of about +56°. The empirical formula of clopidogrel is C_{16}H_{16}ClNO_{2}S and its molecular weight is 321.82.

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![Fig. 3.1 Structural formula of Clopidogrel](image)

Fig. 3.1 Structural formula of Clopidogrel
Table 3.1 Marketed brand name list of Clopidogrel Tablets

<table>
<thead>
<tr>
<th>S.No</th>
<th>Brand Name</th>
<th>Formulation</th>
<th>Strength</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PLAVIX</td>
<td>Tablets</td>
<td>75 mg</td>
<td>Sanofi–Synthelabo (France)</td>
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<tr>
<td>2</td>
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<td>Tablets</td>
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<td>Dr. Reddy’s Laboratories Ltd. (India)</td>
</tr>
<tr>
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<td>Clodrel</td>
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<td>Unichem Laboratories Ltd. (India)</td>
</tr>
<tr>
<td>4</td>
<td>Orawis</td>
<td>Tablets</td>
<td>75 mg</td>
<td>Merck (India)</td>
</tr>
<tr>
<td>5</td>
<td>Noklot</td>
<td>Tablets</td>
<td>75 mg</td>
<td>Zydus Medica (India)</td>
</tr>
<tr>
<td>6</td>
<td>Clopigrel</td>
<td>Tablets</td>
<td>75 mg</td>
<td>USV Ltd. (India)</td>
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<tr>
<td>7</td>
<td>Preva</td>
<td>Tablets</td>
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<td>Intas Pharmaceuticals Ltd. (India)</td>
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<tr>
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<td>Intas Suprima (India)</td>
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<tr>
<td>9</td>
<td>Cloplet</td>
<td>Tablets</td>
<td>75 mg</td>
<td>Sun Pharmaceutical Ind. Ltd. (India)</td>
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<td>Nicholas Piramal (India)</td>
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<td>Torrent Pharmaceutical Ltd. (India)</td>
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3.3 LITERATURE REVIEW

Agrawal et al.,[5] have developed and validated a stability indicating HPTLC method for the determination of clopidogrel bisulphate as bulk drug and in pharmaceutical dosage form. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of carbon tetrachloride/chloroform/acetone (6:4:0.15, v/v). Clopidogrel bisulphate was subjected to acid and alkali hydrolysis, oxidation, photo degradation and dry heat treatment. Densitometric analysis of clopidogrel bisulphate was carried out in the absorbance mode at 230 nm. The linear regression data for the calibration plots showed good linear relationship with $r^2 = 0.9999/0.001$ in the concentration range of 200 - 1000 ng. The mean value of correlation coefficient, slope and intercept were 0.9999/0.001, 0.0939/0.011 and 8.839/0.99, respectively. The method was validated for precision, accuracy, ruggedness and recovery. The limits of detection and quantitation were 40 and 120 ng per spot, respectively. The drug undergoes acidic hydrolysis, basic hydrolysis, oxidative and thermal degradation. However no data were available on the Unknown impurity of our interest.

United States Pharmacopeia-32 (USP-32)[6] has enumerated related substance method for clopidogrel tablets in their monograph, which utilizes liquid chromatography with Ultron ES-OVM L 57, chiral specific column (4.6 mm × 150 mm) packed with 5.0 μ particle size. Acetonitrile and potassium phosphate buffer (10 mM) (75:25, v/v) was used as mobile phase. The flow rate was about 1.0 ml/min and 220 nm used as
wavelength of detection. When analysis was performed by this method the unknown impurity under investigation elutes in the void volume of the system. The known related compounds of clopidogrel were given in figure 3.2.

![Structural Formula of known Related Compounds of Clopidogrel](image)

**Fig. 3.2 Structural Formula of known Related Compounds of Clopidogrel**
Pereillo et al.,[7] have identified and reported that clopidogrel is inactive in vitro and a hepatic biotransformation is necessary to express the full anti-aggregating activity of the drug. Moreover, 2-oxo-clopidogrel has been previously suggested to be the essential key intermediate metabolite from which the active metabolite is formed. In their paper, they have given the evidence of the occurrence of an in vitro active metabolite after incubation of 2-oxo clopidogrel with human liver microsomes. This metabolite was purified by liquid chromatography, and its structure was studied by a combination of mass spectrometry (MS) and Nuclear Magnetic Resonance (NMR) experiments.

Gomez et al.,[8] have made a comparative study with 18 brands of PLAVIX tablets containing clopidogrel hydrogensulfate to the innovator drug product for uniformity of mass, impurity profile, content, dissolution properties and stability. In order to be able to separate the R-enantiomer of clopidogrel, an enantiospecific liquid chromatographic method was used to determine the impurities and to perform the assay. The paddle method was used for dissolution testing. As per the comparative study, most of the brands were not similar compared to the original drug product: their amount of impurities was higher, the content of clopidogrel lower, the dissolution profiles different and after three months under stress conditions in the original packaging, the results for the samples and the reference were significantly different in most of the cases.

Mitakos and Panderi[9] have developed and validated reversed-phase HPLC, stability indicating assay method for the determination of clopidogrel in pharmaceutical dosage forms. The determination was performed on a semi-micro column, BDS C8 (250×2.1 mm i.d., 5 µm particle size); the mobile phase consisted of a mixture of 0.010 M
sodium dihydrogen phosphate (pH 3.0) and acetonitrile (35:65, v/v), pumped at a flow rate 0.30 ml/min. The UV detector was operated at 235 nm and naproxen was used as internal standard. The retention times for clopidogrel and naproxen, which was used as internal standard, were 3.08 and 6.28 minutes respectively. Calibration graphs are linear (r better than 0.9991, n = 6), in concentration range 1.00–3.00 µg/ml for clopidogrel. The intra- and inter-day RSD values were less than 1.96%. Detection and quantitation limits were 0.12 and 0.39 µg/ml respectively.

3.4 SCOPE OF THE STUDY

Literature survey revealed that four impurities of clopidogrel have been already identified\[^5-9\] and named as clopidogrel related compound A, positional stereo isomers of clopidogrel named as clopidogrel related compound B\(_1\) and B\(_2\) and a chiral isomer of clopidogrel named as clopidogrel related compound C. It has also been established that these positional stereo isomers (B\(_1\) & B\(_2\)) are process impurities and other impurities are formed during the process and also self degradation. Marketed samples of plavix and few batches of drug substances were analyzed using reported method\[^6\] and found to contain an unknown impurity. Even though the unknown impurity is formed during oxidation condition, there is no report on the isolation and characterization. Hence comprehensive study was undertaken to identify and characterize the oxidative impurity. The new oxidation impurity is referred to as related compound D.
3.5 EXPERIMENTAL

3.5.1 Materials and Methods

Clopidogrel bisulphate drug substance was purchased from Dr. Reddys laboratories Ltd, Hyderabad, India and clopidogrel tablets of brand name plavix manufactured by Sanofi Pharma Bristol Myers Squibb Inc were used. Potassium phosphate and ammonium acetate, GR grade were obtained from E. Merck, India. Methanol and acetonitrile of HPLC grade were obtained from E. Merck, India. Purified water was collected through Milli-Q water purification system (Millipore, USA). Dimethylsulphoxide-d₆ (DMSO-d₆) was purchased from Aldrich Chemical Co., USA, and all other chemicals used were of analytical grade.

3.5.2 Analytical Method

Analytical method was developed using Waters HPLC system consisting of Alliance integrated hardware of quaternary solvent delivery module, auto sampler and PDA detector. Data were processed through Waters Empower software Version 1.63. Hypersil BDS C8 column (Thermo Electron Corporation) with dimensions of 250 mm × 4.6 mm internal diameter packed with 5.0 µ particle size was employed along with gradient conditions for the separations. The gradient involves two mobile phases consisting of acetonitrile-potassium phosphate buffer (pH 2.3; 10mM) (20:80. v/v) as solvent A and acetonitrile-potassium phosphate buffer (pH 2.3; 10mM) (80:20. v/v) as solvent B. The gradient condition was employed with a timed gradient program of T (min)/%B (v/v): 0.01/0, 5/0, 15/15, 40/30, 45/0, 60/0 for the separations. Flow rate was kept at 1.0 ml/min and the column eluent was monitored at 220 nm for 60 minutes.
3.5.3 Preparative HPLC Method

Preparative HPLC system used was a Waters system equipped with W 600 quaternary solvent delivery module Delta prep 2487 dual wavelength UV detector. Data were processed through Waters empower software. An Xterra MS C18 ODB HPLC column (Waters, Ireland) with dimensions 100 mm × 30 mm packed with 5.0 µ particle size was used for preparative work. The gradient conditions were employed for the separations with a timed gradient program of T (min)/%B (v/v): 01/10, 06/10, 16/95, 17/95, 18/100, 23/100, 28/10, 32/10. Flow rate was kept at 20 ml/min and the column eluent was monitored at 220 nm and 300nm for about 32 minutes.

3.5.4 Infrared spectroscopy

The IR spectra were recorded in the solid state as KBr as dispersion using Shimadzu FT-IR 8700 with DRS technique.

3.5.5 Liquid Chromatography-Mass Spectrometry

The isolated compound was dissolved (about 0.05 mg/ml) in methanol containing 0.1% formic acid (v/v) and infused into the ion source by the syringe pump at the rate of 10µl/min. The mass spectrum of the isolated degradation product was acquired on a Finnegan LCQ instrument from Thermoquest (San Jose CA) in positive spray ionization (ESI+) mode. The spray potential was set at 5.6kV and the capillary temperature at 220°C. Mass range was scanned between 100 and 500amu. The mass spectrum was
also recorded in negative spray ionization (ESI-) mode. The spray potential was set at 5.6kV and the capillary temperature at 220°C.

3.5.6 Nuclear Magnetic Resonance

$^1$H (400.13 MHz) and $^{13}$C (100.62 MHz) NMR spectra of isolated related compound D were recorded on an Avance DPX-400 MHz spectrometer Bruker (Germany). The probe was a $^1$H/$^{13}$C 5 mm, 3 axis gradients (x, y, z), optimized for inverse detection. Spectra were recorded in DMSO-d$_6$ (5-mm tubes) at 300K. Sample concentration was 0.6 mg in 0.6 ml. The residual protonated resonance of the solvent (DMSO-d$_6$) was used as an internal chemical shift standard, which was related to tetramethysilane with chemical shifts of 2.5 and 39.2 ppm, respectively for $^1$H and $^{13}$C. Processing of the raw data was performed using Bruker XWinNmr software. The pulse conditions were 90° pulse, 9.4 µs (attenuation 0db) for $^1$H and 30° pulse, 11.75µs (attenuation 0db) for $^{13}$C. Gradient pulses used in this study were all shaped to a sine envelope with 1 ms duration (DQF-COSY, and $^1$H/$^{13}$C HSQC). Spectral width was 5431.88 Hz for proton and 18111.66 Hz for carbon.

3.5.7 Preparation of degradation samples of Clopidogrel

3.5.7.1 Acid and base degradation

A solution of clopidogrel bisulphate (500mg) in 50ml of 0.1N hydrochloric acid was kept at 80°C for 60 minutes. Another sample was prepared in a similar manner by treating clopidogrel bisulphate (500mg) in 50ml of 0.1N sodium hydroxide.
3.5.7.2 Peroxide degradation

A solution of clopidogrel bisulphate (500mg) in methanol (0.5ml) and hydrogen peroxide (5% in water, 5ml) was kept at 60°C for 3 hours. Similarly 10 tablets of clopidogrel bisulphate were powdered (equivalent to 500mg of clopidogrel bisulphate) and dissolved in 0.5ml of methanol. To the solution, 5 ml of hydrogen peroxide (5%) was added and kept at 60°C for 3 hours.

3.5.7.3 Thermal degradation

Clopidogrel bisulphate (1.0g) was moistened with water and was kept in an oven maintained at 120°C for 24 hours.

3.6 RESULTS AND DISCUSSION

3.6.1 HPLC Method Development and Impurity profiling

An Ultron ES- OVM L 57, chiral specific column (Shinwa chemical industries, Japan) with dimensions of 150 mm × 4.6 mm i.d packed with 5.0 µ particle size was employed for separation. Acetonitrile-potassium phosphate buffer (10 mM) (75:25. v/v) was used as mobile phase and flow rate was kept at 0.8 ml/min with the detection at 220 nm for 30 minutes. Relative retention time (RRT) of the related compound A, B₁, clopidogrel, B₂ and C was found respectively at 0.46, 0.93, 1.0, 1.1 and 2.10. An unknown impurity was found at about 2.0 minutes (RRT of 0.30) (Fig.3.4).
As the unknown impurity elutes in the void volume of the system, various buffer composition, pH, gradients were attempted and found unsuccessful. The main problem that occurred was peak shape, peak purity and blank interference due to peroxide. Hence, conventional, cost effective, new method was developed wherein good peak shape and peak purity were achieved. The new method involves Hypersil BDS C8 column (Thermo Electron Corporation) with gradient conditions for the separations (Fig.3.5).

During the analysis, it has been observed that the new impurity content in clopidogrel tablets, were in the range of 0.05 % to 0.07 % (by area percentage) and in drug substance it ranges from 0.08 % to 0.12 % (by area percentage). Typical chromatograms of clopidogrel drug substance, drug product and drug product spiked with the related compound D were shown in figures 3.6 - 3.8. It is a mandatory requirement from regulatory authorities, to identify and characterize any unknown impurity present in it at a level as low as 0.05% \([7, 8]\). The presence of this impurity in tablets can have a significant impact on quality and safety of the important drug. The isolation of any impurity is required to find the response in an analytical method and also to validate the analytical procedure for its quantitative estimation. Even though the same impurity is formed during oxidation condition, there is no report on the isolation and characterization. Hence comprehensive study was undertaken to identify and characterize the oxidative impurity. The new oxidation impurity in this paper is referred to as related compound D. The chemical structures of related compound D was shown in the figure 3.3.

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Fig. 3.3 Structural Formula of Clopidogrel related compound D
Fig. 3.4 Typical HPLC chromatogram of Clopidogrel spiked with Impurities as per USP
Fig. 3.5 Typical HPLC chromatogram of Clopidogrel spiked with Impurities as per developed
Fig. 3.6 HPLC chromatogram of clopidogrel drug substance
Fig. 3.7 HPLC chromatogram of Clopidogrel drug product
Fig. 3.8 HPLC chromatogram of Clopidogrel drug product spiked with related compound D
3.6.2 Analysis of degradation samples by analytical LC

The degradation samples were diluted to the required concentration and analyzed with analytical LC. In the oxidative conditions, 2% and 7% of related compound D was found in the drug substance and drug product (plavix) respectively. In other conditions, formation of related compound D was not noticed. The purity angle of all the impurities was found less than that of purity threshold and thus the peak purity of the impurities was confirmed. The spectrum of the related compound D and clopidogrel were extracted from PDA detector in the range of 210 to 400 nm. The UV spectra are presented in the figure 3.9. Hence the experiment was used to enrich the impurity. It was also confirmed that related compound A and C are the degradation products and related compound B₁ and B₂ are process impurities based on their trend. The higher level of related compound D in the drug product reveals that the drug product is more susceptible than that of drug substance.

3.6.3 Isolation of degradation product(s) by preparative LC

Clopidogrel bisulphate (5g) was treated with hydrogen peroxide (5%, 15ml) and kept at 80 °C for 3 hours. The aqueous layer was washed with dichloromethane to remove clopidogrel. The aqueous layer was subjected to preparative LC as described in the section 3.5.3 and as many as 10 fractions were collected separately. Purity of all these fractions was analyzed by analytical LC and found to be in the range of 99%. The fractions were pooled together, 100mg of sulfuric acid was added and the solvent was evaporated. The resulted solid was reanalyzed on analytical LC and the purity of the
same was found to be 99% which was good enough for carrying out the spectroscopic experiments.

### 3.6.4 Characterization of the degradation product

The isolated related compound D was injected in both the analytical HPLC methods. The retention time and UV spectrum obtained in the PDA detector matches with that of targeted impurity. Characterization of the related compound D was performed using analytical data obtained from CHNS, IR, UV, and Mass, MSn experiments, $^1$H /$^{13}$C NMR spectrum, DEPT and 2D NMR experiments. The Mass, MS-MS and IR spectrum are given in figures 3.10-3.13.
Fig. 3.9 UV spectral overlay of Clopidogrel and Related Compound D
Fig. 3.10 MS-MS spectrum of Related compound D ($^{35}$Cl isotope)
Fig. 3.11 MS-MS spectrum of Related compound D (Cl$^{37}$ isotope)
Fig. 3.12 IR spectrum of Related compound D
Fig. 3.13 Mass spectrum of Related compound D
The isolated related compound D was found as off white powder and it shows UV absorbance maxima at 299.9 nm which is higher than that of clopidogrel (220 nm). The +ve ES-MS spectrum of the related compound D showed peaks at m/z 320 and 322 corresponding to the $^{35}\text{Cl}$ and $^{37}\text{Cl}$ isotope respectively. The compound doesn’t form lithium adduct ion and –ve ES-MS spectrum showed no peaks states that the molecular ion obtained is positively charged i.e. m/z 320/322 is due to $\text{M}^+$. In comparison with clopidogrel, the related compound D corresponds to 1 atomic mass unit (amu) less which can be presumed to have similar structure as that of clopidogrel but with short of one hydrogen atom.

Two daughter ions were obtained at m/z 183 and 155 when the molecular ion ($\text{M}^+$) 320 fragmented in MS/MS experiments. Both the daughter ions were found to contain chlorine atom as it was confirmed by MS/MS experiments of the chlorine isotope molecular ion ($\text{M}^+$) 322 and the m/z values of the daughter ions were 185 and 157. As the peak intensity ratios are nearly identical, it was confirmed that the eliminated neutral fragments contains no chlorine atom. Further MS$^2$ experiments of daughter ion (m/z 183) showed the formation of a fragmentation ion at m/z 155 and MS$^3$ shows the formation of an ion at m/z 125. Similar experiments were performed with the $^{37}\text{Cl}$ Isotope of daughter ion of m/z 185 and fragmentation ions of m/z 157 and 127 were found. Based on the fragmentation data, the structure for related compound D was assigned as shown in the figure 3.3 and its probable fragmentation pathway is given in the figure 3.14.
Fig. 3.14 Fragmentation pattern of related compound D
The fragmentation pathway of related compound D was compared with the clopidogrel fragmentation pathway given in the figure 3.15.\textsuperscript{[12]} It was found that m/z value of the clopidogrel daughter ion is 212/214 but the same was not observed in the case of related compound D. The fragmentation ion obtained from MS\textsuperscript{2} experiments of clopidogrel was 183/185 and 155/157 which is similar to that of the daughter ion of related compound D. Similar fragmentation was not observed in related compound D, due to the double bond associated with nitrogen atom. However the fragmentation ion obtained from MS\textsuperscript{2} experiments of clopidogrel was similar to that of the daughter ion obtained from related compound D. This was confirmed by MS\textsuperscript{3} and MS\textsuperscript{4} experiments.

\textsuperscript{1}H NMR spectrum of related compound D is slightly different from that of clopidogrel \textsuperscript{1}H NMR spectrum and exhibits one hydrogen less than that of clopidogrel. Comparison of \textsuperscript{1}H, \textsuperscript{13}C and DEPT 135 NMR data with that of clopidogrel (Table 3.2 and 3.3, Fig. 3.16-3.18) shows that the related compound D has one methylene proton less and one methine proton more than that of clopidogrel. The methine protons at 2\textsuperscript{nd} and 3\textsuperscript{rd} positions are deshielded to the extent of 0.3, 0.7 ppm respectively.

The methylene protons at 6\textsuperscript{th} and 7\textsuperscript{th} positions are deshielded to the extent of 0.5, 0.4 ppm respectively. The presence of methylene protons at 6\textsuperscript{th} and 7\textsuperscript{th} positions were confirmed by irradiation (proton decoupling) experiment (Fig.3.19). Irradiation of protons at chemical shift $\delta$ 4.30 ppm (m) affects the multiplicity of protons at $\delta$ 3.94 and 3.80 ppm which further reveals the double bond position. The appearance of one singlet at $\delta$ 9.19 ppm at 4\textsuperscript{th} position and the deshielding of methine proton at 10\textsuperscript{th} position from
δ 5.60 to δ 6.66 ppm were also noticed. All the above deshielding confirmed that there was a structural change in the piperidine ring. It was also confirmed by $^1$H-$^1$H COSY spectrum. There is no appropriate change in the chemical shift of aromatic ring which confirms the presence of the aromatic rings in the related compound D. $^{13}$C and DEPT 135 NMR spectra results indicate the presence of one methyl carbon, two methylene carbons, eight methine carbons and five quaternary carbons. Related compound D shows five quaternary carbons like that of clopidogrel but one methylene carbon less and one methine carbon more.

Fig. 3.15 Fragmentation pattern of Clopidogrel bisulphate
Table 3.2 $^1$H and $^{13}$C NMR assignments for clopidogrel bisulphate and related compound

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<th>Position</th>
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<td></td>
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<td>J (Hz)</td>
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<td>–</td>
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$^a$ Refer structural formula (Fig. 1) for numbering: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad.

$^b$ Unresolved.
The methylene signal at δ 49.62 ppm disappeared and a new methine signal at δ 162.51 ppm was observed. The high deshielding chemical shift value of the methine signal indicates that carbon may be attached to an electronegative atom or attached to aromatic ring or under the anisotropic influence of an aromatic ring. This led to the hypothesis of existence of C=N bond in the piperidine ring of related compound D structure. The position of the double bond was assigned to 4\textsuperscript{th} carbon due the possibility of extended conjugation explained for the shifting of UV absorbance maxima to the higher wavelength. This was also confirmed by the deshielding of aliphatic methine carbon at 10\textsuperscript{th} position to about 5 ppm and the deshielding of one of the quaternary carbon at 8\textsuperscript{th} position to about 20 ppm. The presence of C=N bond was further confirmed by the IR characteristic absorption peak at 1469 cm\textsuperscript{-1}.

All the above observations can be explained well on the basis of the proposed structure (Fig.3.3) with quaternary nitrogen and a double bond. All proton signals were assigned on the basis of \textsuperscript{1}H NMR and \textsuperscript{1}H-\textsuperscript{1}H COSY spectral results. Carbon signals were assigned on the basis of DEPT135 and \textsuperscript{1}H-\textsuperscript{13}C HSQC spectral results. Based on the above spectral data, the structure was characterized as 5-[1-(2-chlorophenyl)-2-methoxy-2-oxoethyl]-6, 7-dihydrothieno [3, 2-c] pyridin-5-ium with a molecular weight of 320 amu.
Fig. 3.16: H NMR Spectrum of Clopidogrel Related compound D
Fig. 3.17 $^{13}$C NMR Spectrum of Clopidogrel Related compound D
Fig. 3.18 DEPT 135 NMR Spectrum of Clopidogrel Related compound
3.7 CONCLUSION

The oxidative degradation product, related compound D in clopidogrel drug substance as well as drug product was isolated by preparative LC and was characterized by using spectroscopic techniques namely NMR, CHNS, IR, MS and MS$^n$. 

Fig. 3.19 Irradiation data (Proton decoupling experiment)
REFERENCES


