Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2010, 2(4): 244-252
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

Non chiral High Performance Liquid Chromatography method for monitoring unknown impurities generated during stability of Clopidogrel tablets

P. Aparna[a*], Sumathi V. Rao[a], K. M. Thomas[a], K. Mukkanti[b] and Kishore Deo[c]

[a*, a ,c,] *APL Research Centre, Bachupally, Hyderabad, India*
[b] *Department of Chemistry, J. N. T. University, Kukatpally, Hyderabad, India*

ABSTRACT

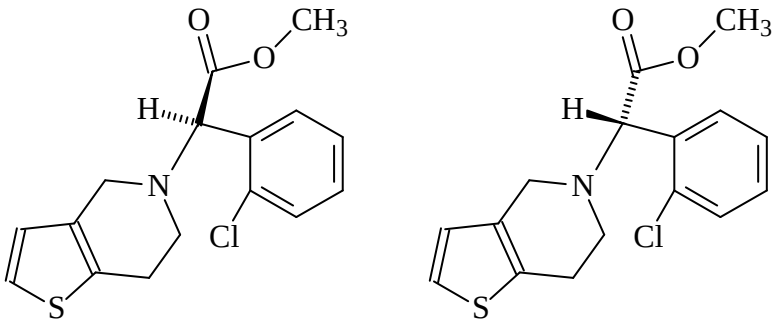
The purpose of this study was to develop a High performance liquid chromatography (HPLC) method for separating the unknown impurities generated during the accelerated stability storage of Clopidogrel Bisulphate Tablets. Also, we used the newly developed method to identify the factors that contribute to the formation of these unknown impurities in the tablet formulation. Study was carried out by incubation of mixture of excipients and Clopidogrel API in 5:1 ratio at 80°C for 3days. The new HPLC method was developed by using Kromasil 100 C18 column and using gradient method with mobile phase of 0.1% Triflouroacetic acid in water in pump A and 0.1% Trifluoroacetic acid in Acetonitrile in pump B was suitable for separating the unknown impurities from the Clopidogrel Related Compound A. The method discussed in United States Pharmacopoeia (USP) is not suitable to separate these impurities. From the excipients compatibility data we hypothesized that these unknown impurities were generated due to the excipient Polyethylene Glycol that is present in the tablet both as a tablet lubricant as well as a part of the film coating system. Further these unknown impurities were characterized as Dihydro pyridinone Derivative, Decarbmethoxylated Clopidogrel.

Keywords: Clopidogrel, Impurities, Stability, HPLC.

INTRODUCTION

Clopidogrel is an anti-platelet agent, which selectively inhibits the binding of the adenosine diphosphate to its platelet receptor and blocks the subsequent activation of the glycoprotein complex thereby inhibiting platelet aggregation. As shown in **Fig.1** the molecule is a thienopyridine derivative containing asymmetric carbon leading to the existence of two enantiomer the R and S form and more active form of Clopidogrel is S enantiomer.

Fig. 1



a. Clopidogrel R- Enantiomer b. Clopidogrel S- Enantiomer

The current USP monograph refers to three related substances, related compound A, B and C for the drug substance and Clopidogrel Related Compound A and C for the drug product and specifies limits for the same [1-2]. Our stability studies on the Clopidogrel tablets showed cluster of impurities getting generated after 1M of incubation at 40°C/75% RH and this increases in a non-linear fashion during 2M and 3M storage. The current USP method is not able to successfully separate these impurities. Our preliminary study indicated that this might be due to the fact that the USP method is a chiral method while the impurities generated were probably non chiral in nature and highly polar. The literature search did not throw up a satisfactory method to monitor such impurities [3-9].

The aim of our study was to develop a reverse phase HPLC method that would successfully separate the unknown impurities generated during a stability run and also to understand which factor is most likely to contribute to the development of the impurities in Clopidogrel Tablet formulations.

MATERIALS AND METHODS

2.1 Manufacturing of Tablets:

The core tablets formulation contains Clopidogrel Bisulphate (Aurobindo Parma Ltd) and excipients like Microcrystalline cellulose (FMC Bio polymer), Mannitol (Roquette), Low substituted hydroxyl propyl cellulose-11, Low substituted hydroxy propyl cellulose-21(Shin-Estu chemical co Ltd), Crospovidone (ISP Technologies Inc), Hydrogenated castor oil (Cognis), Polyethylene glycol 6000 (Clariant), Opadry pink (Colorcon Asia Pvt. Ltd). Manufacturing process essentially consist of three steps, slugging of the drug and excipients, breaking the slug into granules and compression into tablets and film coating the tablets. The tablets were packed in white HDPE (High density poly ethylene) containers and were incubated at 40˚C 75% RH (Relative Humidity) sampling was done at 1 month, 2 month following incubation.

2.2 Drug-Excipients Mixtures:

The drug and each of the excipients were separately mixed in 1:5 ratio and packed in HDPE containers. The containers were incubated for 3 days at 80˚ C and these samples were analyzed by the developed HPLC method.

2.3 High Performance Liquid Chromatography:

A Waters 2695 separation module equipped with 2996 photodiode array detector with empower pro data handling system (Waters corporation, Milford, MA, USA) was used.

2.4 NMR Spectroscopy:
The ^{1}H NMR spectra were recorded on Bruker 300MHz nuclear magnetic resonance spectrometer using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard.

2.5 Mass Spectrophotometer:
Mass spectra were recorded on PE SCIEX-API 2000 mass spectrometer equipped with a turbo ion spray interface at 375°C. Detection of ions was performed in electro spray ionization positive ion mode.

2.6 Samples:
The investigated samples, Clopidogrel tablets were formulated in APL Research center (A unit of Aurobindo Pharma Limited, Hyderabad, India). All reagents used for analysis i.e. Trifluoroacetic acid (GR Grade), Acetonitrile, Methanol (HPLC Grade), were procured from Merck (India) Limited. Milli-Q water was used for the analysis.

2.7 Sample preparation:
The finely powder of clopidogrel tablets was taken equivalent to 100mg of Clopidogrel into a 100 ml dry volumetric flask, 5ml methanol added and shake for 5 minutes. Further 50ml of diluent again shake for 30minutes diluted to volume with diluent and mixed. Filtered the solution through 0.45 membrane filter.

2.8 Diluent preparation:
Water and Acetonitrile mixed in the ratio of 80:20 v/v

RESULTS AND DISCUSSION

3.1 Development of the Method:
Initial analysis was carried out by using the USP method (**Fig 2a**). However when we analyzed the 1M, 2M and 3M accelerated samples, a cluster of unknown impurity peaks eluting very near to the related compound A peak (**Fig: 2b-d**) were observed. The level of these impurities appeared to be just above identification threshold levels (identification threshold level for Clopidogrel tablets is 0.2%). So, there is a need to modify the USP method to separate these unknown impurity peaks satisfactorily. The reason may be due to the fact that the USP method is based on Chiral separation and the unknown impurities may not be chiral in nature and are definitely more polar than Clopidogrel as well as both of its reported related compounds A & B. We developed a Reverse Phase HPLC method using, Kromasil 100 pack $C_{18,}$ 250 mm column 4.6 mm i.d, 5 µ particle diameter column (FLEXIT). Mobile phase A containing 0.1% Trifluoroacetic acid in water (1 ml of Trifluoroacetic acid in 1000 ml water), Mobile phase B containing 0.1% Triflouro acetic acid in Acetonitrile (1 ml of Triflouro acetic acid in 1000 ml Acetonitrile). UV detection was carried out at 220 nm and flow rate was kept at 1.0 ml/minutes, column oven temperature was set at 45 °C and data acquired for 35 minutes. The following time programme gave the best separation for all three unknown impurities from Clopidogrel Related compound A: Time (Min)/A (v/v):B(v/v) $T_{0.01}$/80:20 T_{25}/50:50 T_{26}/80:20 T_{35}/80:20.This method was run on the LC-MS to identify these impurities. By using this developed HPLC method, the cluster of three impurities were getting resolved, which were observed as cluster in the USP method.

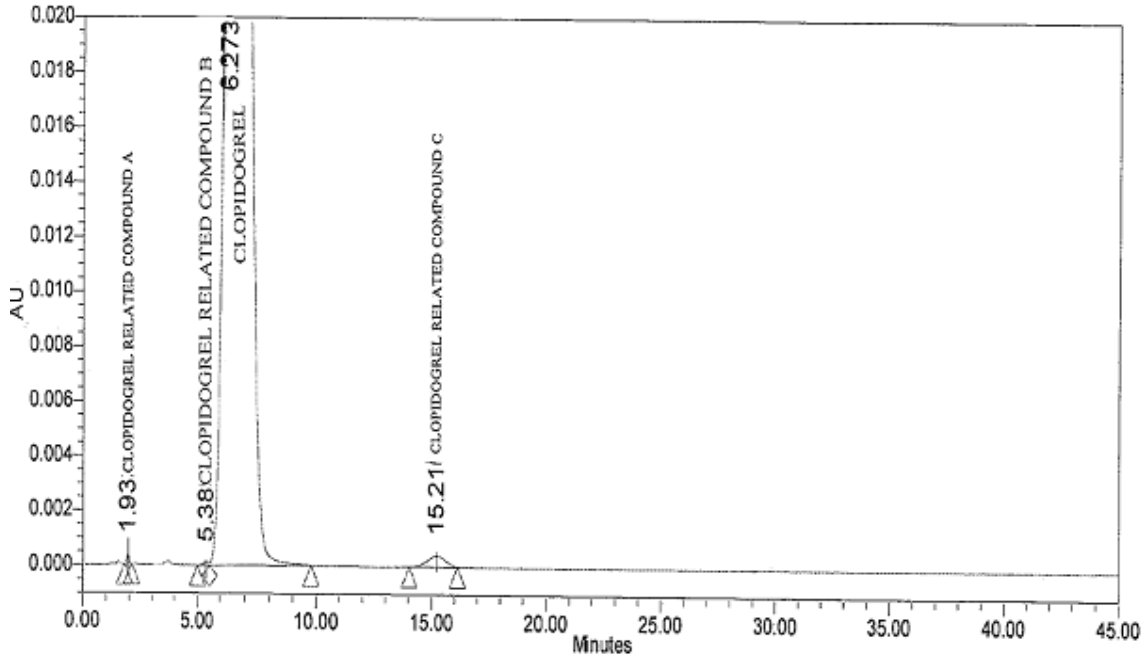


Fig- 2.a Initial sample chromatogram by USP method

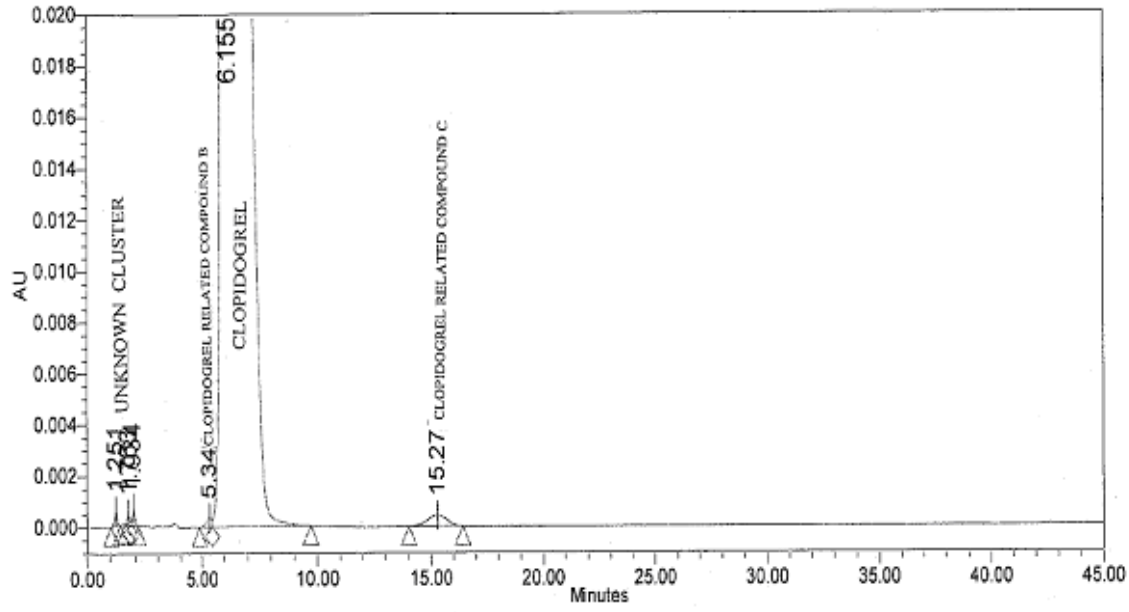


Fig- 2.b. 1 Month stability sample Chromatogram by USP method

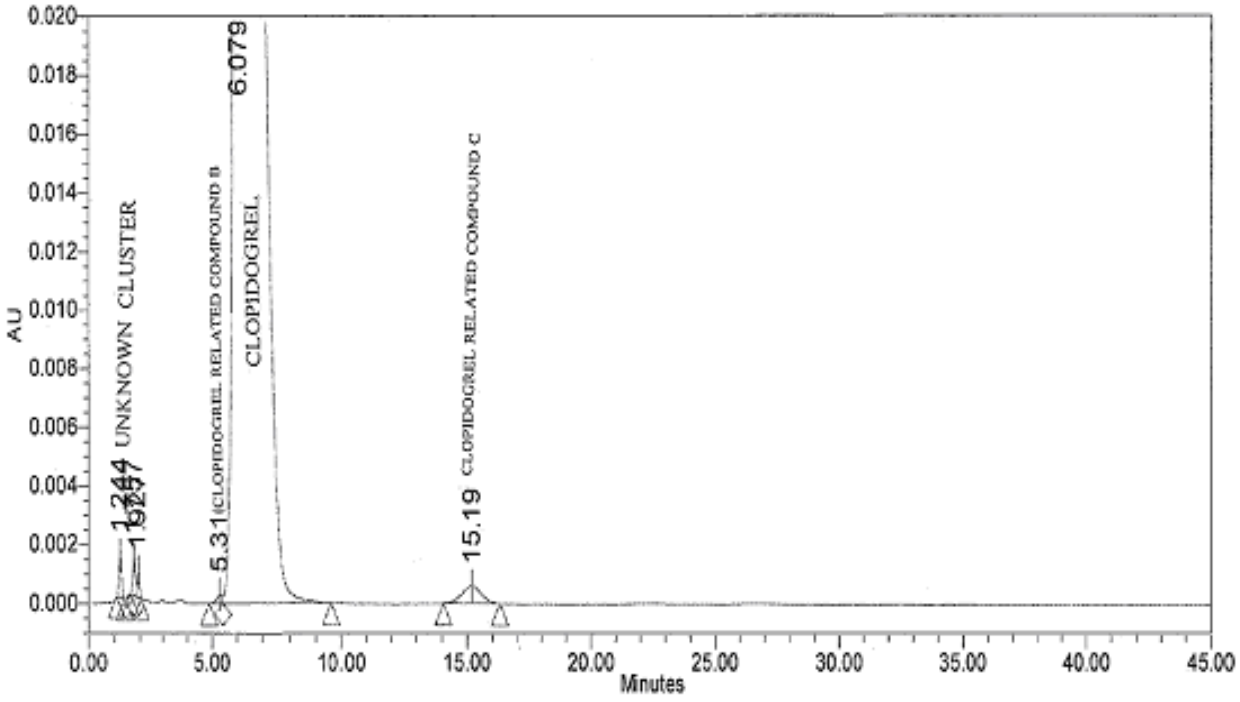


Fig- 2.c. 2 Month stability sample Chromatogram by USP method

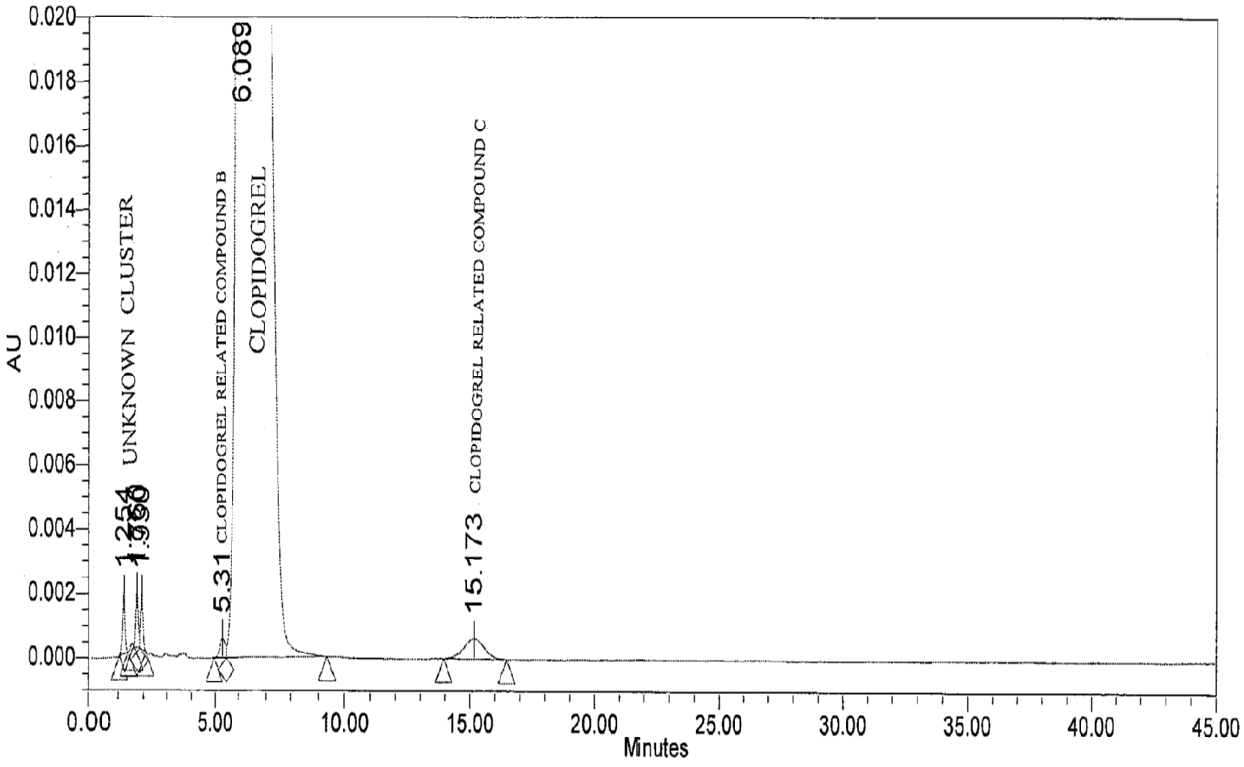


Fig- 2.d. 3 Month stability sample Chromatogram by USP method

3.2 Stability Studies:

The initial, 1Month, 2 Month and the 3 Month accelerated sample analysis was performed using the above developed method and the chromatograms are shown in **Fig 3a-d**. From the data presented in **Fig 2a-d** it is observed that it is not possible to separate all the unknown impurities, which can generate during a stability study by the chiral-based HPLC method. Hence, we

attempted to develop a simple reverse phase method, which will separate out all these impurities. The method described above and the **Fig 3a-d** show that by our method it is possible to separate all the three unknown impurities which were eluting as a cluster by the USP method. This may be because our method is not chiral based and the generated impurities are highly polar in nature. **Fig 3c** and **Fig 3d** show that these impurities increase on further incubation at 2M and 3M indicating that the developed method is able to discern the change in the level of these impurities.

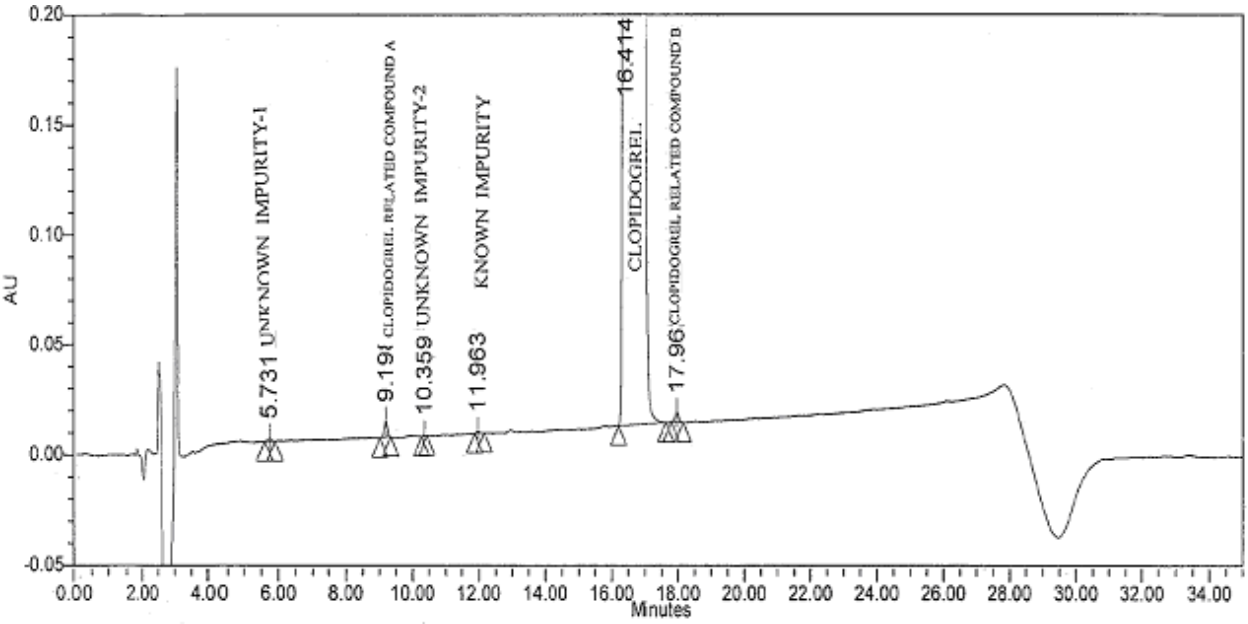


Fig- 3.a. Initial sample Chromatogram by newly developed method

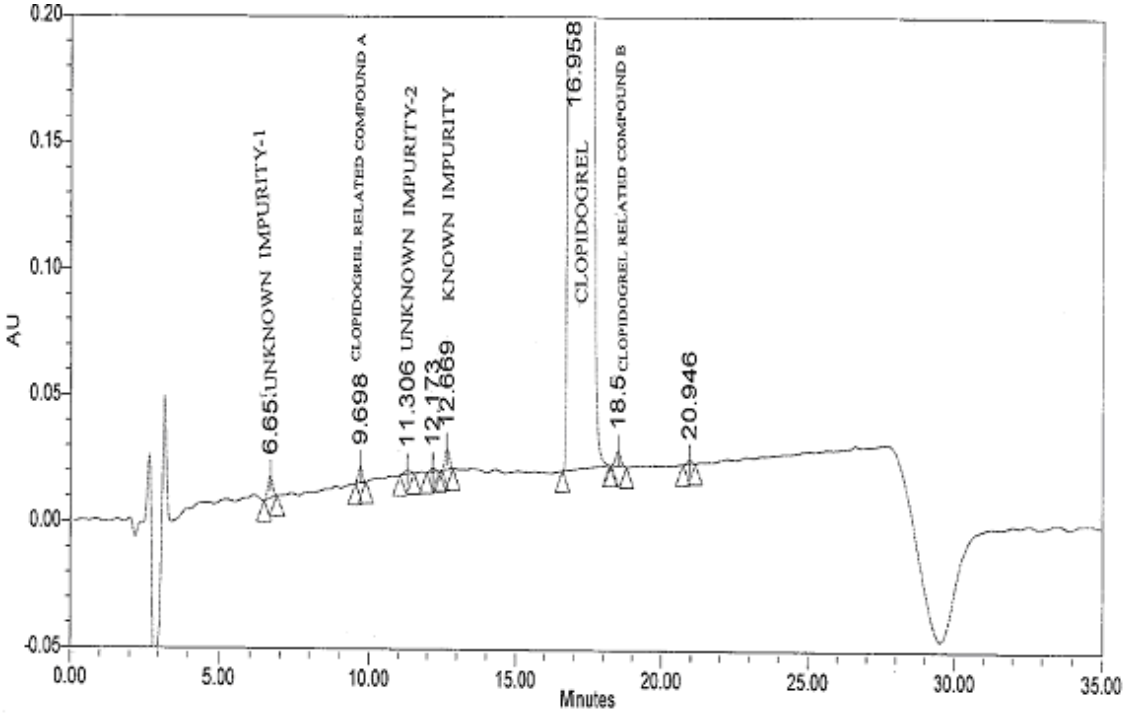


Fig- 3.b. 1 Month stability sample Chromatogram by newly developed method

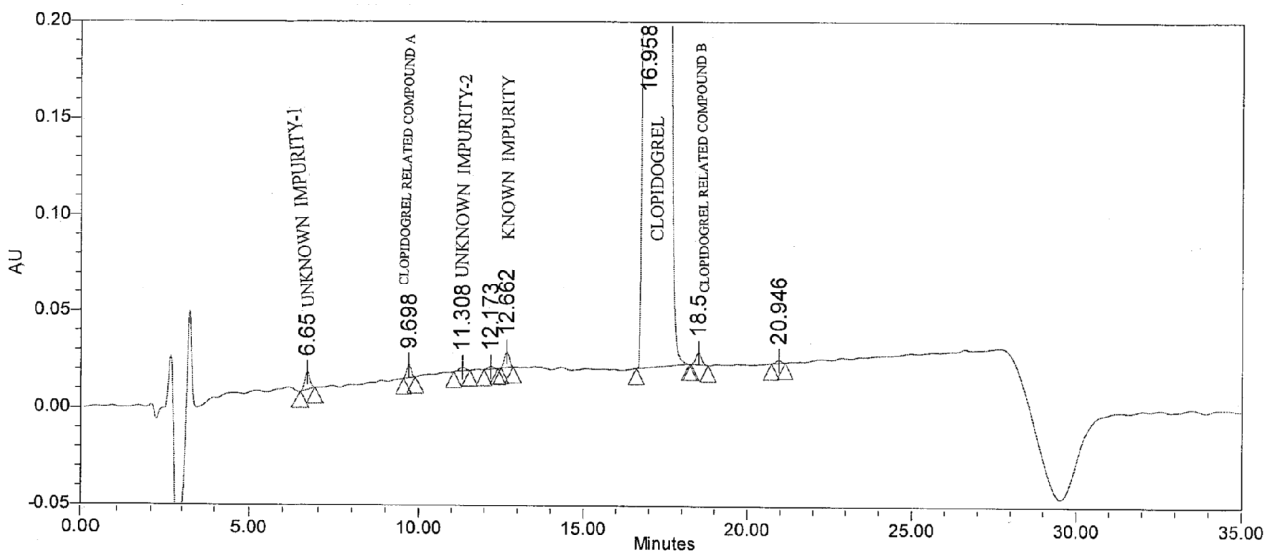


Fig- 3.c. 2 Month stability sample Chromatogram by newly developed method

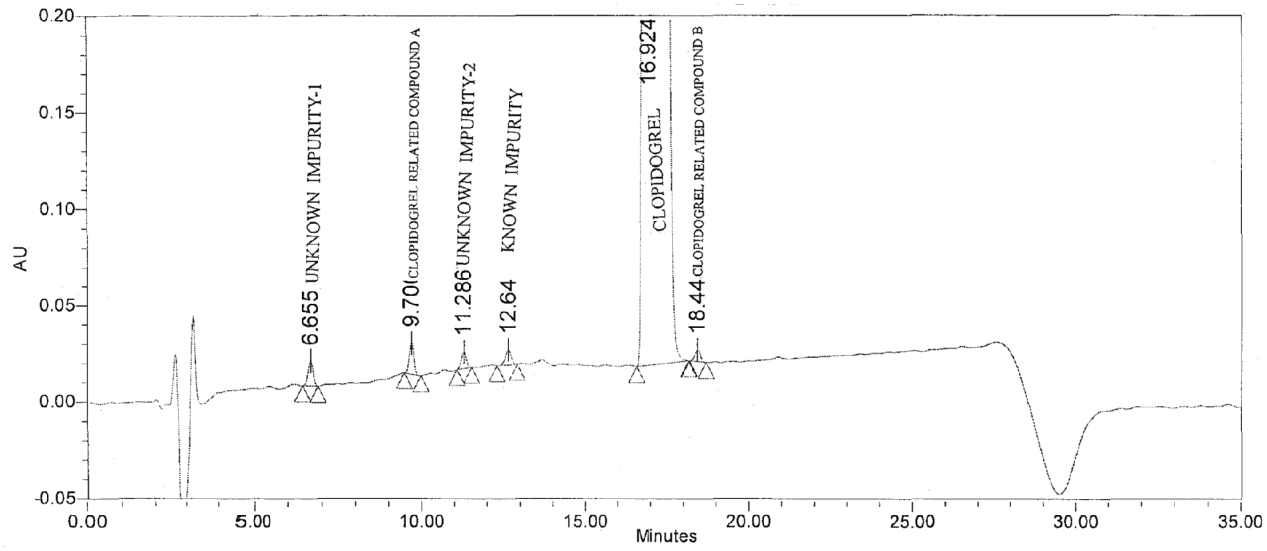


Fig- 3.d. 3Month stability sample Chromatogram by newly developed method

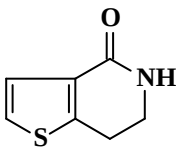
The drug and excipients physical mixture analysis was carried out using the developed method and the results are recorded in (**Table 1**). The excipients compatibility data **Table-1** indicates that polyethylene glycol which is present in the core tablets as a lubricant and which is also present in the film coating system is most probably responsible for the generation of these unknown impurities. At 1:5 ratio of drug and these excipients, the level of the impurities generated is significant. However, at the actual use level in the final formulations, the level of impurities is found at around 0.3% level.

Table –1. Excipients compatibility data

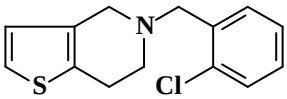
Drug and Excipients	% Unknown Impurity – I at RRT 0.35		% Unknown Impurity - II at RRT 0.65		% Unknown Impurity – III at RRT 0.74	
	Controlled	80°C-3 Days	Controlled	80°C-3 Days	Controlled	80°C-3 Days
Microcrystalline cellulose	0.01	0.02	0.01	0.02	0.01	0.03

Mannitol	0.00	0.01	0.00	0.00	0.01	0.02
Low substituted hydroxy propyl cellulose-11	0.01	0.01	0.00	0.01	0.01	0.01
Low substituted hydroxy propyl cellulose-21	0.00	0.01	0.00	0.01	0.01	0.01
Crospovidone	0.00	0.01	0.00	0.02	0.01	0.02
Hydrogenated castor oil	0.00	0.00	0.00	0.00	0.01	0.05
Polyethylene glycol	0.01	0.03	0.00	0.46	0.01	0.62
Opadry pink	0.01	0.01	0.00	0.28	0.01	0.06

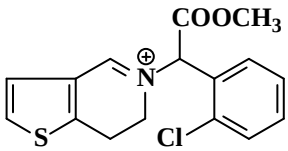
In order to identify and characterize the impurities, we ran the stability samples by the developed method using preparative HPLC, collected the fractions eluting out at Relative Retention Time (RRT) 0.35, 0.65 and 0.74 and subjected these fractions to LC-MS. The m/z values were found to be 153,264 and 321 respectively. The impurities at RRT 0.35 was identified and characterized as dihydropyridinone derivative and the other impurity at RRT 0.65 was decarbmethoxylated Clopidogrel and that of another impurity at RRT 0.74 was identified as a known impurity i.e. methyl ester of Dihydropyridinone-2 [10]. The structures of these impurities are shown below:



Dihydro pyridinone Derivative (Un known imp-1 at RRT 0.35)



Decarbmethoxylated clopidogrel (Un known imp-2 at RRT 0.65)



Methyl ester of Dihydropyridinone-2 (known imp at RRT 0.74)

The Impurities discussed in literature mainly focused on the degradation impurities pertaining to clopidogrel bisulphate drug substance. However, the stability study performed on clopidogrel tablets as per the actual ICH conditions reveal that apart from methylester of Dihydropyridinone-2, Dihydropyridinone derivative and decarbmethoxylated clopidogrel were also observed as degradation impurities. These impurities have been subjected to invitro toxicity studies, which is underway.

CONCLUSION

The current chiral-based HPLC method official in the USP is not capable of separating all the impurities generated during the accelerated stability run for Clopidogrel Tablets. The reverse phase HPLC method developed by us is able to separate our all the three unknown impurities indicating that the method is better suitable as a stability indicating method for Clopidogrel Tablets. We were able to identify and characterize the impurities formed during stability. The probable formulation ingredient responsible for generation of these impurities has also been identified as polyethylene glycol. The two unknown impurities were identified and characterized as Dehydro pyridinone derivative and Decarbmethoxylated Clopidogrel and the third one was identified as known impurity of Methyl ester of Dihydropyridinone-2 [10].

Acknowledgement
The authors gratefully acknowledge the management of Aurobindo Pharma Limited, in general and APL Research Centre in particular for allowing us to carry out the present work. The authors are also thankful to the colleagues of Analytical research department.

REFERENCES

[1] The United States of Pharmacopoeia 32-edition, pg 1992.
[2] The United States of Pharmacopoeia 32-edition, pg 1993.
[3] Y. Gomez, E.Adams, J. Hoog martens, *J. Pharm. Biomed. Anal*., **2004**, 34, 341-348.
[4] Himani Agarwal.Neeraj Kaul, A.R. Paradkar, K.R.Mahadik, *Talanta* **2003**, 61, 581-589.
[5] A.Mitakos, I.Panderi, *J. Pharm. Biomed. Anal*., **2002**, 28, 431-438.
[6] H.Y.Aboul-Enein, H. Hoenen, A.Ghanem, M. Koll, *J. Liq. Chrom. Rel. Technol.*, ***2005***, 28, 1357-1365.
[7] A. Robinson, J.Hillis, C.Neal, A.C.Leary, *J.Chromatogr. B: Anal. Tec. Biomed. Life Sci*., **2007**, 848, 2, 344-354.
[8] E.Souri, H.Jalalizadeh, A.Kebriaee-Zadeh, M.Shekarchi, A.Dalvandi, *Biomed. Chromat*., **2006**, 20, 1309-1314.
[9] H.Ksycinska, P.Rudzki, M.Bukowska-Kiliszek, *J. Pharm. Biomed. Anal*., **2006**, 41, 533-539.
[10] K.Dhara Raijada, Bhagwat Prasad, Amrit paudel, P.Ravishah, Saranjit Singh, *J. Pharm. Biomed. Anal*., **2009**, 52, 332-344.