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Development of a Validated Stability Indicating RP-HPLC Method for Assay of Clopidogrel

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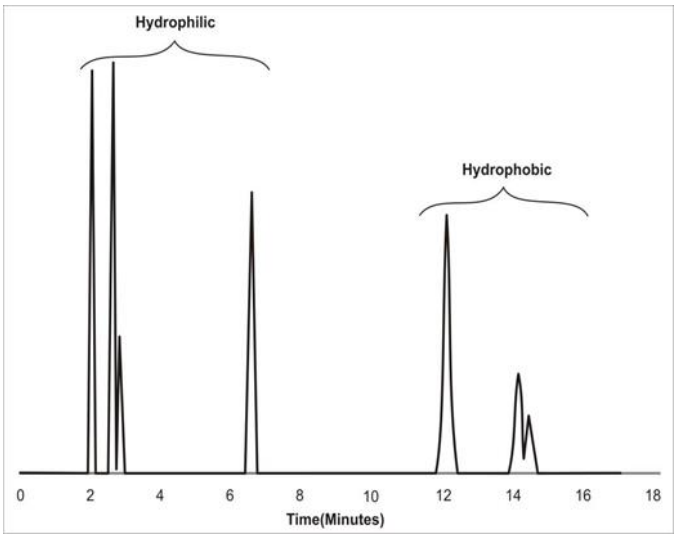
ABSTRACT

A simple, precise, rapid, selective, and economic reversed phase high-performance liquid chromatography (RP-HPLC) method has been established for estimation analysis of DRO. A Brownlee ODS C-18 column (250×4.6 mm i.d) chromatographic column equilibrated with mobile phase methanol-0.02 M KH2PO4 (80:20, v/v) (Final pH adjusted to 4 using Orthophosphoric acid) was used. Mobile phase flow rate was maintained at 1 ml/min and effluents were monitored at 254 nm. The sample was injected using a 20 µl fixed loop, and the total run time was 9.946 min. Experimental conditions such as pH of mobile phase, column saturation time, selection of wavelength, etc. were critically studied and the optimum conditions were selected. In RP-HPLC linear range was found to be 1-10 µg/ml and, mean recovery was found to be 99.65 and Rt of Clopidogrel was found to be 9.946 min.

Keywords : Clopidogrel, Validation, RP-HPLC

I. INTRODUCTION

Reversed phase HPLC is characterized by a situation in which the mobile phase used is MORE POLAR than the stationary phase. The name 'Reversed Phase' arises as this was the second, (chronologically), mode of chromatography after Normal (or 'Straight') phase in which a polar stationary phase is used in conjunction with a less polar mobile phase. Typical reversed phase stationary phases are hydrophobic and chemically bonded to the surface of a silica support particle. Other support materials and bonded phases are available



Representative reversed phase chromatogram detailing analyte retention order based on hydrophobicity or Hydrophilicity

For neutral analytes, the mobile phase consists of water (the more polar component) and an organic modifier that is used to vary the retention of analytes by lowering the polarity of the mobile phase. The

most common organic modifiers are shown. Increasing the water content will repel ('squeeze') hydrophobic (non-polar) analytes out of the mobile phase and onto the non-polar stationary phase where they will reside for a time until 'partitioning' out into the mobile phase again. Each 'on-off' partition is called a 'Theoretical Plate'. When ionizable (or ionic) analytes are present, other additives such as buffers or ion pairing reagents can be added to the mobile phase to control retention and reproducibility. The Chromatogram illustrates the general elution order of hydrophilic and hydrophobic analytes. When working with ionizable analytes the hydrophobicity and, hence, retention characteristics of the analyte will be affected depending on its ionization state (ionized or non-ionized), this will be discussed later in the module.

II. EXPERIMENTAL WORK

❖ **Standard solutions**

- **Solution A (Stock standard solution)**

Accurately weighed quantity of Clopidogrel (10.0 mg) was dissolved in methanol to make 10.0 ml solution. (conc.: 1.0 mg/ml)

- **Solution B (Working standard solution)**

Accurately measured 1.0 ml of solution A was diluted to 100.0 ml with mobile phase (conc.: 10.0 □g/ml).

- **Preparation** of buffer

10 mM Phosphate buffer was prepared by dissolving 13.609 g of Potassium dihydrogen phosphate in 100.0 ml of milli-Q water and 1.0 ml of resulting solution was diluted to 100.0 ml with milli-Q water and pH was adjusted to 6.5 with 0.1 M NaOH.

❖ **Optimization of chromatographic conditions**

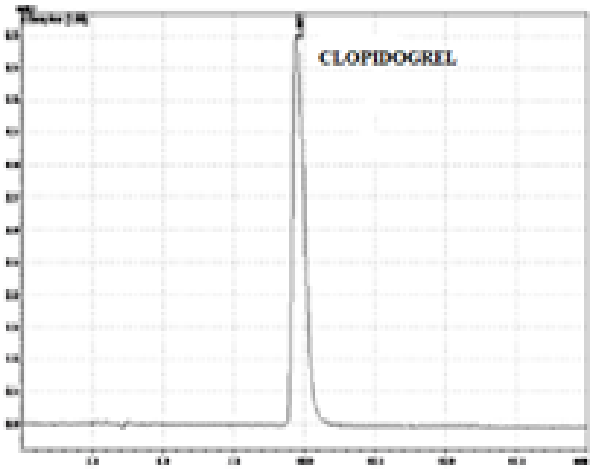
The chromatographic studies were performed on C8 analytical column. Initially, different mobile phases were tried in isocratic mode to get an adequate retention of Clopidogrel. Mobile phase containing methanol: water, methanol: ammonium acetate was tried, but retention time of Clopidogrel was about 2.25 min. Then acetonitrile: water was tried, but retention time of Clopidogrel was about 3.56 min and peak was separated. Then acetonitrile: sodium hydrogen orthophosphate was tried, retention time of Clopidogrel was about 12.69 min but with excessive peak tailing and there was a large baseline noise. Further, acetonitrile: potassium dihydrogen phosphate (10 mM, pH 6.5 with 0.1 M NaOH) (10: 90 *v/v*) was tried which gave a retention time of 9.946 ± 0.039 min with flow rate of 1.0 ml/min. The same mobile phase when tried for forced degradation samples, Clopidogrel was found to be well resolved from its degradation products.

Hence, the mobile phase consisting of acetonitrile: potassium dihydrogen phosphate (10 mM, pH 6.5 with 0.1 M NaOH) with the ratio of 10: 90 *v/v* was selected as an optimum mobile phase which gave good resolution of Clopidogrel from its degradation products along with good peak symmetry and retention of drug at about 9.946 □ 0.039 min.

The maximum absorption wavelength of reference drug and forced degradation samples was found to be 254 nm and hence selected as detection wavelength for analysis.

Following are the optimized chromatographic conditions for further study:

Column:	**Enable C8 column (250 x 4.6 mm, 5 µm)**
Mobile phase :	**Acetonitrile: Potassium dihydrogen phosphate buffer, (10 mM, adjusted to pH 6.5 with 0.1 M NaOH), 10:90 *v/v***
Detection Wavelength:	**254 nm**
Injection volume:	**20 µl (Rheodyne injector)**
Flow rate:	**1.0 ml/min**
Temperature:	**250 C (Room Temperature)**



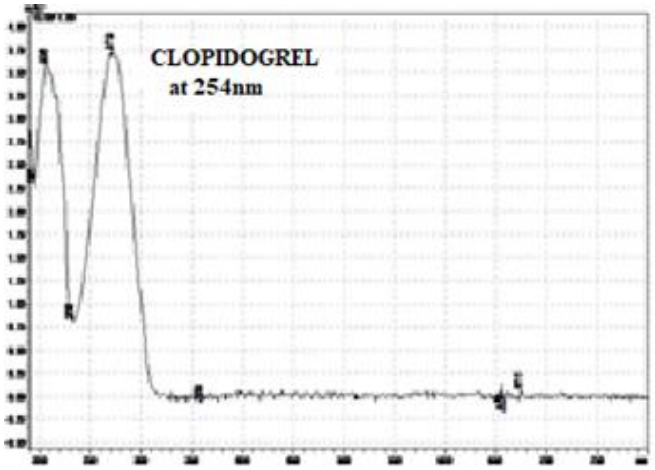
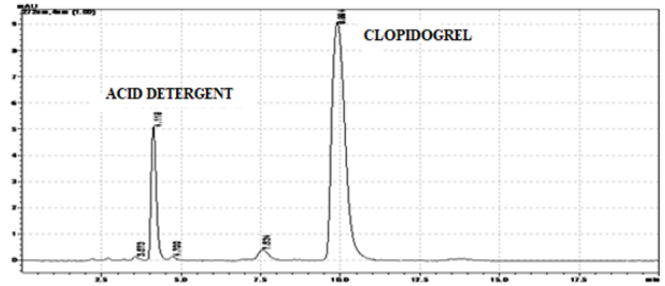
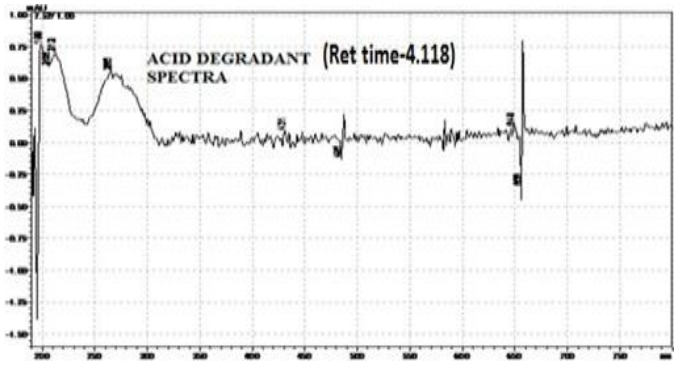


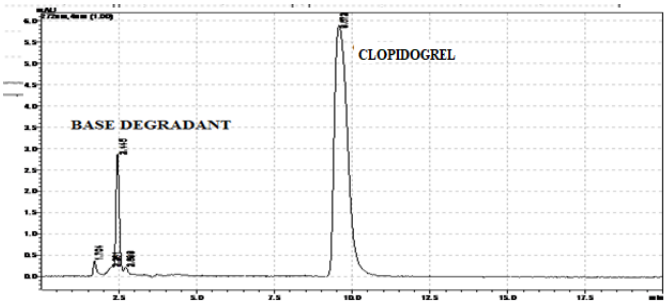
Figure: (a) HPLC chromatogram and (b) *in situ* spectrum of Clopidogrel

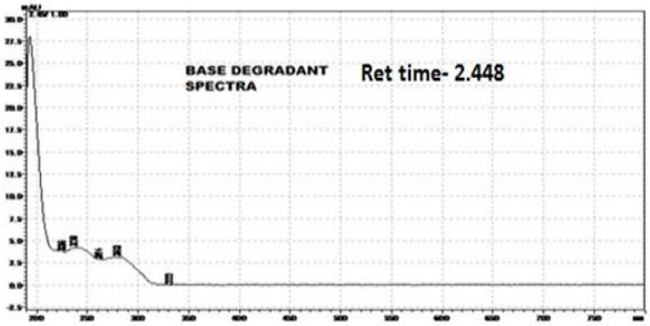
All the stability samples were analyzed to study the extent of degradation and if the reasonable degradation (5-20%) with respect to parent drug has seen, the stress testing was stopped at that point.



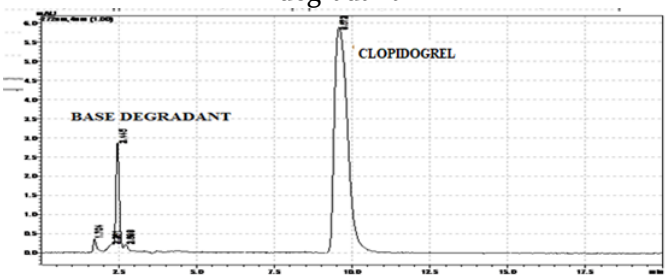


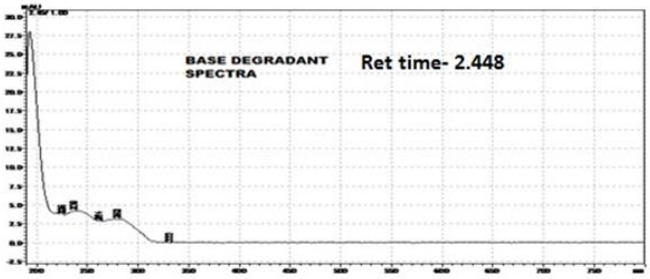
A) Acid (1M HCL, 24 h reflux) and Spectra of degradant



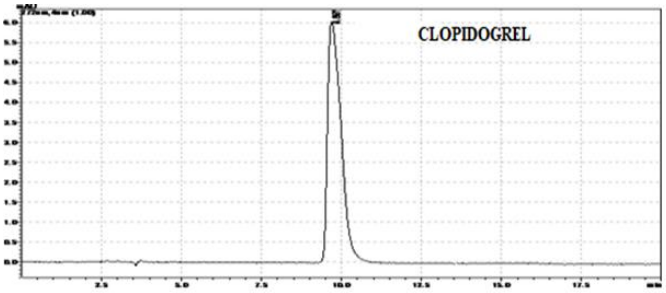


B) Base (1M NaOH, 24 h reflux) and Spectra of degradant

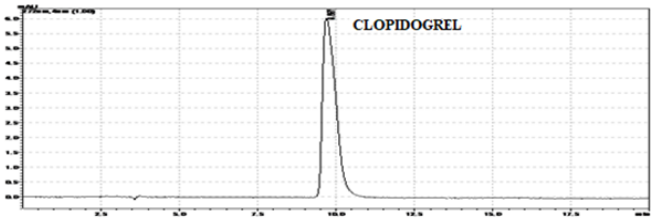




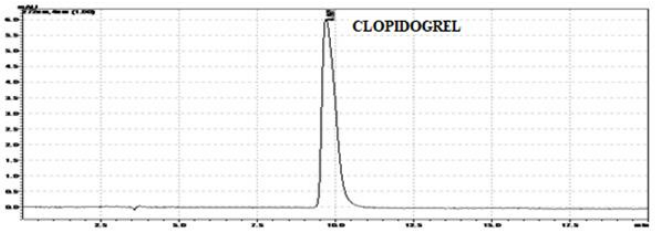
C)Neutral Stress, 24h reflux



D)Oxidative Stress (3% H2O2, 48 h at R.T.)



E)Photolytic Stress, (15 days, 4 lac reflux h)



F) Thermal Stress, (dry heat, 15 days at 700C)

Figure 1 : HPLC chromatograms of forced degraded samples (A-F)

Chromatogram of mixed degradation products

Chromatogram was also obtained under similar chromatographic conditions for mixed degradation products.

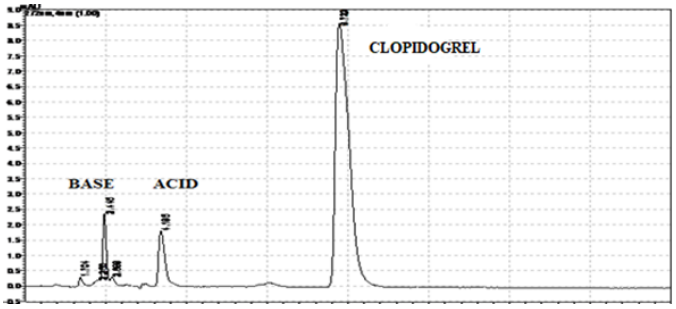


Figure 1 : Chromatogram of mixed degradation product

❖ Study of system suitability parameters

The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase to get steady baseline. Five replicate injections of working standard solution B were made separately and the chromatograms were recorded.

Table : Study of system suitability parameters

Sr. No	Retention Time (min)	Asymmetry	No. of Theoretical plates	Capacity Factor	Peak Area	Peak height
1	9.894	1.267	6597.560	1.769	126335	6391
2	9.932	1.252	6653.906	1.810	125598	6387
3	9.969	1.232	6674.371	1.804	126089	6288
4	9.939	1.231	6616.350	1.854	126370	6404
5	9.996	1.230	6622.484	1.826	126119	6396
Mean	**9.946**	**1.244**	**6632.934**	**1.813**	**126102.20**	**6373.20**
± SD	**0.039**	**0.017**	**30.791**	**0.031**	**308.459**	**48.049**
%RSD	**0.389**	**1.386**	**0.464**	**1.717**	**0.245**	**0.754**

Table: Study of system suitability parameters

Linearity of response

Aliquot portions of standard solution B (1.0, 2.0, 3.0, 4.0, 5.0, 6.0 & 10.0 ml) were diluted to 100.0 ml with mobile phase to get concentration 1-6.0 µg/ml. The chromatographic condition were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase to get the steady baseline. Prepared standard solutions of different concentration were injected separately and the chromatograms were recorded.

A graph was plotted as peak area vs. concentration of drug (µg/ml)

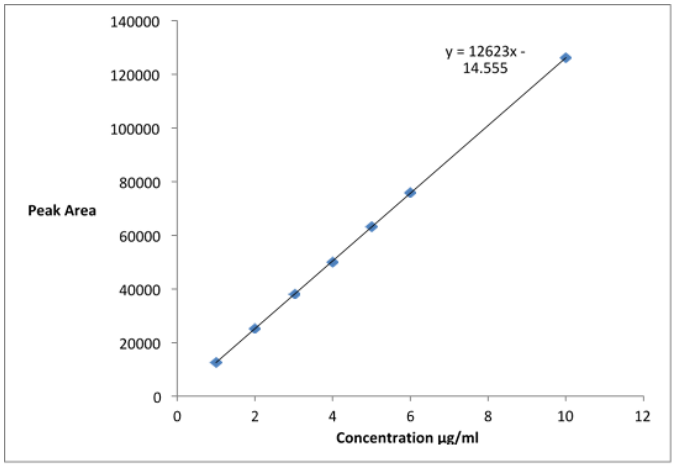


Table: Results of Linearity studies

Concentration range	1.0- 10.0 µg/ml
Equation for straight line	Y= 12623X-14.55
Slope	12623
Y-intercept	(-) 14.55
Correlation coefficient	1

III. REFERENCES

[1]. Nadig, D. E., "Preparation of drug sample for Analysis", Handbook of Pharmaceutical Analysis; Ohannesian, L., New Jersey, 2002; p. 1-3.

[2]. Sonia K, Beddi Bhavya shree, Dr.K.S.Lakshmi, "HPTLC Method Development and Validation: An Overview", J. Pharm. Sci. & Res. Vol. 9(5), 2017,652-657

[3]. Vishal N. Kushare, Sachin S Kushare, Development of Validated Stability Indicating HPTLC Method for Assay of Ozagrel and its Pharmaceutical Formulations, InternationalJournal of Scientific Research in Science, Engineering and Technology. November-December-2018 [4 (11): 36-48]

[4]. Vishal N. Kushare, Sachin S Kushare, Sagar V Ghotekar, Development of Validated UVSpectrophotometric Method for Assay of Ozagrel and its Pharmaceutical Formulations. .International Journal of Scientific Research in Science, Engineering and Technology,November-December-2018 [4 (11): 69-77]

[5]. Dr.D.S.Ghotekar, Kushare Vishal N, Characterization and Evaluation of Warfarin (Anticoagulant Drug) in Immediate Release Tablet Dosage Form Journal of Emerging Technologies and Innovative Research (JETIR)Volume 5, Issue 12,December 2018. pg. no- 204 to 212

[6]. Vishal N Kushare, Sagar V Ghotekar. Development of a Validated Stability Indicating RP-HPLC Method for Assay of Ozagrel and Its Pharmaceutical Formulations, International Journal of Pharmacy and Pharmaceutical Research. January 2019 Vol.: 14, Issue: 2.pg.no 47-65

[7]. Dr.D.S.Ghotekar Vishal N. Kushare ,Validated Stability Indicating HPTLC of Clopidogrel and its Pharmaceutical Formulations.International Journal of Scientific Research in Science, Engineering and Technology. January-February-2019 [6 (1) : 557-567]

[8]. Dr.D.S.Ghotekar Vishal N. Kushare , Assay of Clopidogrel by Using HPLTC Method.International Journal of Scientific Research in Science and Technology. (www.ijsrst.com) November-December-2018 [4 (11) : 557-567]

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