INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

DEVELOPMENT OF A VALIDATED HPLC-PDA METHOD FOR STABILITY INDICATING STUDY OF TICAGRELOR: A NOVEL ANTI-PLATELET AGENT ($P2Y_{12}$-ADP RECEPTOR BLOCKER)

Md. Shabana Sulthana[1], V. Anuradha[2] and Mandava V Basaveswara Rao[3]*

[1]Department of Chemistry, Acharya Nagarjuna University, Guntur Dist, Andhra Pradesh, India.
[2]Department of Chemistry, Vignan Degree & PG College, Guntur Dist, Andhra Pradesh, India.
[3]Department of Chemistry, Krishna University, Krishna Dist., Andhra Pradesh, India.

ABSTRACT

A HPLC method for the estimation of direct acting and reversible $P2Y_{12}$-ADP receptor blocker, Ticagrelor and its stability indicating study was developed and validated according to ICH guidelines. Stress testing was carried out under aqueous, acidic, alkaline, oxidative, photolytic and thermolytic conditions. The degradation was observed in alkaline and oxidative conditions and the drug remained stable in other applied conditions. Good elution pattern was achieved on X-Select CSH Phenyl-Hexyl column (150mm X 4.6mm i.d., 3.5µm particle size) using mobile phase comprising of 70% methanol in water. Reference compounds of these degradants were obtained using semi preparative HPLC purification. Degradants were characterized by Mass spectrometry and Nuclear magnetic resonance spectroscopy. The formed degradants are confirmed to be de-Ethylated and diasteriomeric N-oxide products of ticagrelor. The developed method was validated for linearity (10-60 µg/mL), precision (RSD ≤ 3.893%), accuracy (recovery 98 – 101%) and sensitivity (LOD 0.1µg/mL). This method is simple and convenient for the assay of ticagrelor in dosage form and its stability indicating study.

Keywords: Ticagrelor, HPLC, Stability indicating study.

INTRODUCTION

Ticagrelor (TCG), (1S,2S,3S,5S)-3-[7-[(1R,2S)-2-(3,4 Difluorophenyl)cyclopropyl-amino]-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxy)-cyclopentan-e-1,2-diol, an anti platelet agent with novel mechanism of action that involves to inhibit prothrombic effect of ADP by blocking platelet $P2Y_{12}$ receptor and this effect is reversible and this non-competitive antagonist of $P2Y_{12}$ receptor resulting in no receptor activation in spite of increased ADP concentration[1,2]. TCG offers potential advantages in the treatment of anti platelet therapy in patients with acute coronary syndrome or myocardial infarction and in experimental studies that were shown to increase ADP response by shifting dose response curve for adenosine induced coronary blood flow velocity to the left[3]. Evident pharmacodynamic effects were observed during the transitions from intravenous cangrelor to oral ticagrelor and vice-versa but there was no significant interaction between the binding of cangrelor and ticagrelor[4]. But there was a higher number of fatal intracranial bleeding, raised abdominal pressure and spontaneous omental bleeding[5-7].

There are reports in previous literature for the determination of unbound TCG and its active metabolites in human plasma by dialysis using LC-MS and LC-MS/MS[8, 9]. Stability indicating LC-UV method[10] was reported for the determination of TCG in Brilinta formulation. However structural elucidation studies of

degradants and validation of HPLC method has not been carried out. According to ICH guidelines[11] the developed HPLC method should be validated for the API and also the degradants formed under various stress conditions[12, 13]. Presented work describes purification, characterization (NMR, MS) of the three degradants and development of a validated HPLC-PDA method for stability indicating analysis and the assay of TCG in Brilinta formulation.

EXPERIMENTAL

Reagents and Chemicals

HPLC grade Methanol (Lichrosolv) purchased from Merck, Mumbai and ultra purified water passed through 0.45 µ obtained using Merck Elix essential 3 water purification system (Millipore – USA). Hydrochloric Acid (HCl) (26% w/w), Sodium hydroxide (NaOH) pellets and Hydrogen peroxide (H_2O_2) (AR Grade) 30% solution were purchased from Rankem Chemicals (Mumbai), Dimethyl sulfoxide- d_6 purchased from Sigma-Aldrich (St. Louis, MO, USA). BRILINTA™ tablets (90 mg of TCG per each tablet) were purchased from local market (manufactured by AstraZeneca Operations, SE-151 85, Sweden, Lot: TDAG, Expirary date: 01 -2015). TCG was obtained as a gift sample from Rainbow labs, Hyderabad.

Instrumentation

Analytical liquid chromatography

HPLC system equipped with quaternary pump ultimate 3000 model (Thermo scientific), auto sampler of ASI-100 model (50µL injection loop) and photo diode array detector PDA-100. Mobile phase consist of 70% Methanol in water (solvent A: water, solvent B: methanol) in isocratic mode at the flow rate of 1 mL.min^{-1}. Reliable Separation was achieved on Waters X Select CSH Phenyl-Hexyl (150mm X 4.6mm, 3.5µm) column. Eluent was monitored at 298nm with PDA detector. The control of HPLC system and data processing was performed using Chromeleon 6.80 series version.

Semi preparative liquid chromatography

For the purpose of obtaining pure form of degradants semi preparative HPLC was carried out equipped with Gilson 321 binary pump (Middleton, WI, USA), GX-281 liquid handler and UV-155 detector. Mobile phase in the same combination of solvents and Waters X select CSH Phenyl Hexyl (150mm X 10mm, 3.5µm) was used for fractionation. Flow rate was maintained at 4 mL min^{-1}. Eluent was monitored at 298 nm. Trilution-LC 2.3 software was used for data processing.

NMR analysis

In the process of structural elucidation ^{1}H NMR, ^{13}C NMR and DEPT NMR measurements were performed on 300 MHz Avance 500 NMR spectrometer (Fallanden, Switzerland). DMSO-d_6 was used as solvent.

Degradation experiments

For the purpose of stability indicating studies degradation of TCG was carried out under various stress conditions. The process of degradation is shown below.

Acid degradation studies

100mg of TCG was added to 5mL MeOH and refluxed with 5mL 2M Hydrochloric acid at 80^{0}C for 5h. The solution was neutralized with NaOH.

Alkali induced degradation study

100mg of TCG was added to 5mL MeOH and refluxed with 5mL 2M Sodium hydroxide at 80^{0}C for 5h then neutralized with HCl.

Oxidative induced degradation study

Accurately weighed 100 mg of TCG dissolved in 5mL MeOH and 5mL H_2O_2 10% (v/v) and then refluxed under 80^{0}C for 5h. The solution was diluted with MeOH.

Photolytic degradation study

Accurately weighed 50mg of TCG subjected to daylight for 72h. Then the solution was diluted with MeOH to optimal concentration.

Dry heat induced degradation study

50mg of TCG was subjected to dry heat at 70^{0}C for 72h. All these solutions were fractionated using semi preparative chromatography for obtaining pure form of each degradant and the purified degradants was subjected to spectroscopic studies for their characterization.

HPLC Analysis

Mobile phase consists of 70% Methanol in water in isocratic mode at the flow rate of 1 mL.min^{-1}. Reliable Separation was achieved on Waters X Select CSH Phenyl-Hexyl (150mm X 4.6mm, 3.5µm) column. Eluent was monitored at 298nm with PDA detector.

Standard solutions

1 mg/mL stock solutions were prepared by dissolving 1mg of TCG, DG-1, DG-2 and DG-3 individually in 1mL of MeoH. Stock solutions were further diluted with methanol to obtain concentration in the range of 20-70 µg mL^{-1} and 5-25 µg mL^{-1} for TCG and degradants respectively.

Sample solutions

Six tablets of Brilinta formulation were homogenized and an aliquot was prepared to obtain the concentration equivalent to 1 mg/mL of TCG and filtered through 0.45 µ PTFE. This solution was further diluted to the level of concentration 50 µg/mL with methanol.

RESULTS AND DISCUSSIONS

Identification of degradation products

After following all the protocol methods for degradation of TCG it is understood that TCG was found to be degraded in alkaline and oxidative conditions only. In all other conditions TCG remained stable. From the structural studies major degradant was confirmed as de Ethylated product of TCG which is as per IUPAC (1S,2R,3S,4S)-4-(7-((1R,2S)-2-(3,4-difluorophenyl)-cyclopropylamino)-5-(propylthio)-3H-[1,2,3]-triazolo[4,5-d]pyrimidin-3-yl)cyclopent-ane-1,2,3-triol from the alkaline degradation and the other two degradants formed in oxidative degradation were confirmed as diasteriomeric mixture of N-oxide form of TCG in 1:1 ratio. The possible degradation pathway of TCG in NaOH (2N) and Hydrogen peroxide (10% v/v) is given in Fig. 1.

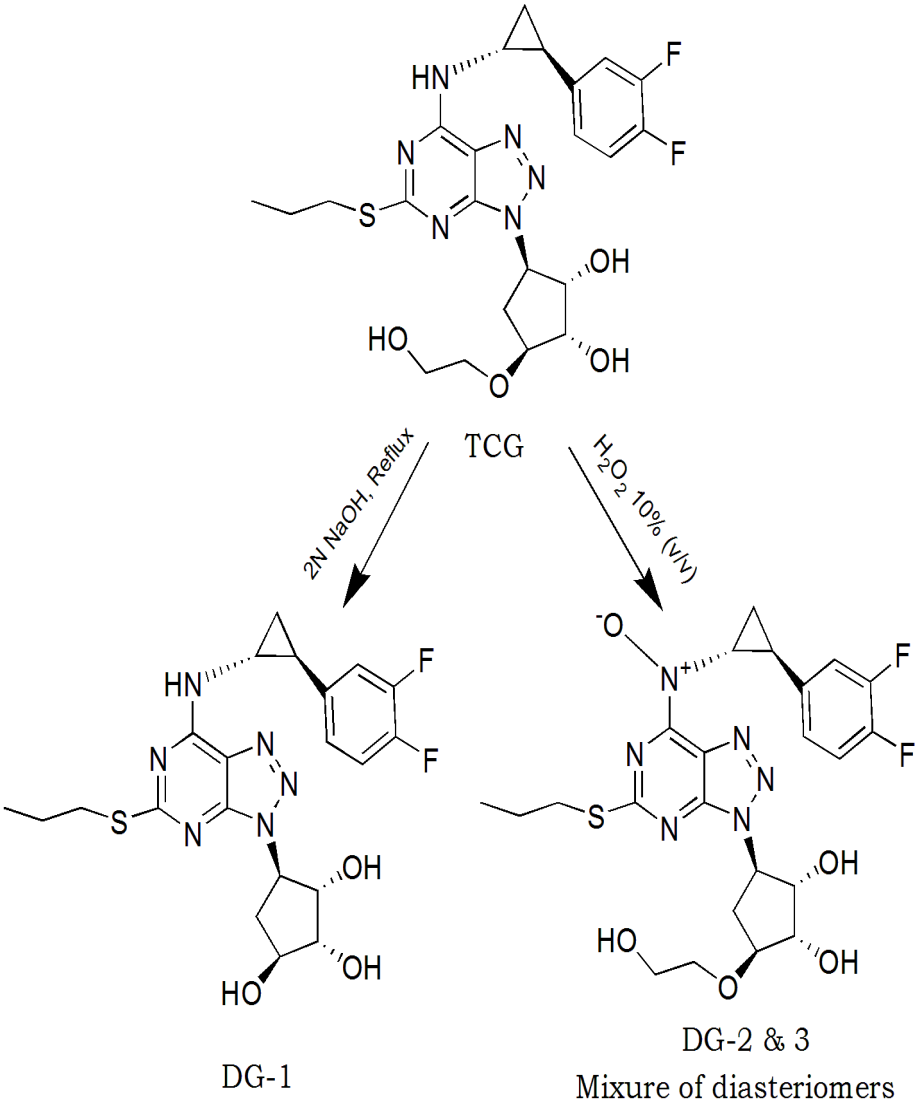

Fig. 1: Suggested pathway for the degradation of TCG in 2N NaOH and 10% H_2O_2

Spectral data of the formed degradants is presented below

De Ethylated TCG

^{1}H NMR (DMSO-$_{d6}$ 300 MHz): 8.84 (d, 1H), 7.70-7.52 (m, 2H), 7.10-7.00 (m, 1H),4.62(1H,m), 3.95 (brs, 1H), 3.82-3.78 (m, 1H), 3.59-3.49 (m, 4H), 3.25-3.10 (m, 1H), 2.90-2.81 (m, 2H), 2.62 (d, 1H), 2.25-2.22and 2.13-2.05 (m, 1H), 2.03-2.01 (m, 1H), 1.73-1.38(m, 4H), 0.98 (t, 3H). Calc HRMS 501.1491, Observed 501.1519 $(M+Na)^+$.

N-Oxides of TCG

^{1}H NMR (DMSO-$_{d6}$ 300 MHz): 9.84 (brs, 1H), 7.37-7.27 (m, 2H), 7.02 (m, 1H), 5.18 (brs, 1H), 5.09 (d, J=9.0 Hz, 1H), 5.08 (q, 11H), 4.67 (m, 1H), 4.56 (m, 1H), 3.97 (brs, 1H), 3.77 (m, 1H), 3.51 (m, 4H), 3.20-3.09 (m, 1H), 2.95 (m, 1H), 2.84 (m, 1H), 2.68 (m, 1H), 2.15 (m, 1H), 2.01 (m, 1H), 1.71-1.33 (m, 4H), 0.87 (t, j=7.3 Hz, 3H). Calc HRMS 539.1883, Observed 539.1895 $(M+H)^+$.

From the structural studies it was confirmed that N-Oxide of TCG exists as mixture of diasteriomers in 1:1 ratio.

Method validation

The optimized HPLC method was validated according to ICH guidelines for TCG and its degradants **[14]**. All the parameters such as assay, linearity, accuracy, precision (intra & inter day), detection limits, quantification limits, robustness and system suitability were evaluated.

Linearity

The linearity was evaluated by analyzing at different concentration levels of each compound. Six series of concentration levels ranging 1-100 µg mL^{-1} and 5-25 µg mL^{-1} for TCG and degradants respectively were prepared and analyzed. The linearity data was given in Table 1.

LOD and LOQ

Detection and quantitation limits were determined by calculating S.D of the response and slope. The results are provided in Table 1.

Table 1: Characteristic parameters of Ticagrelor and its degradation products

Parameter	TCG	DG-1	DG-2	DG-3
Calibration range (µg mL^{-1})	20-70	5-25	5-25	5-25
Detection limit (µg mL^{-1})	0.611	0.868	0.250	0.537
Quantitation limit (µg mL^{-1})	2.038	2.896	0.833	1.793
Standard Error	0.419	0.056	0.104	0.083
Slope	0.828	0.090	0.207	0.222
SD of slope	0.009	0.003	0.001	0.001
%RSD of slope	1.197	3.684	0.903	0.562
Intercept	-9.668	-0.056	0.179	0.125
SD of intercept	0.168	0.026	0.017	0.039
Correlation coefficient	0.9990	0.9998	0.9997	0.9998

Selectivity

Selectivity of the method was evaluated by injecting blank, standard mixture containing TCG and its degradants, sample and placebo individually. No peak was observed in HPLC chromatogram for blank and placebo indicating no interference from the excipients commonly used in the tablet formulations.

System suitability

The system suitability parameters which includes resolution (*Rs*), asymmetry (*A*), sensitivity (*S*), theoretical plates (*N*), skewness, relative retention time (*RRT*) and % relative standard deviation (*% R.S.D*) were assessed and the results were represented in Table 2.

Table 2: System suitability parameters of the proposed HPLC method

Analyte	Avg Rt (min)	Resolution (Rs)	Asymmetry	Sensitivity (mAU)	Theoretical plates	Skew-ness	Relative Retention Time	%RSD of Rt
TCG	10.88	-	1.05	0.001	14075	1.02	-	0.17
DG-1	4.10	11.37	0.99	0.001	2025	0.96	37.68	0.22
DG-2	4.71	7.25	1.03	0.001	2500	1.04	43.29	0.56
DG-3	5.25	7.37	1.12	0.001	2500	1.10	48.25	0.60

Precision

Intraday and inter day injections were performed in triplicate for TCG and its degradants to assess the precision of the method which is calculated as % R.S.D. The % R.S.D values for TCG and its degradants were found to be in the range of 0.22-2.59 for intraday and 0.86-2.80 for inter day which is acceptable limits and represented in Table 3.

Table 3: Intra-day and inter-day precision and accuracy of the method

Analyte	Intra Day (n=3)		Inter Day (n=3)	
	Conc (µg mL^{-1}) ± SD	%RSD	Conc (µg mL^{-1}) ± SD	%RSD
	20 ± 0.19	2.593	20 ± 0.15	2.199
TCG	50 ± 0.46	1.432	50 ± 0.61	2.271
	70 ± 0.48	0.993	70 ± 0.93	1.500
	5 ± 0.01	0.867	5 ± 0.01	1.701
DG-1	15 ± 0.03	1.758	15 ± 0.04	2.803
	25 ± 0.06	2.656	25 ± 0.05	1.569
	5 ± 0.02	1.904	5 ± 0.01	0.868
DG-2	15 ± 0.02	0.227	15 ± 0.08	2.685
	25 ± 0.09	1.735	25 ± 0.05	1.028
	5 ± 0.03	1.951	5 ± 0.03	1.885
DG-3	15 ± 0.02	0.833	15 ± 0.04	1.326
	25 ± 0.04	0.746	25 ± 0.08	1.641

Accuracy

Accuracy study was performed by the standard addition method. Results indicate that the accuracy was within the acceptable limits and represented in Table 4.

Table 4: Recovery studies

Amount added (µg mL^{-1})			
Analyte	1.25	1.5	1.75
% Recovery ± %RSD			
DG-1	101.13 ± 0.164	103.42 ± 0.174	99.79 ± 0.84
DG-2	102.98 ± 0.544	102.61 ± 0.153	104.94 ± 0.608
DG-3	103.14 ± 0.593	101.18 ± 0.315	100.36 ± 0.19
Amount added (µg mL^{-1})			
	50	75	100
% Recovery ± %RSD			
TCG	102.77 ± 1.227	101.50 ± 0.930	101.26 ± 0.987

Application of validated LC method for the estimation of TCG in Brilinta tablets

Developed HPLC method was applied to quantify the amount of TCG in Brilinta tablets. Satisfactory results were obtained for TCG in a good agreement with the label claims. Typical chromatogram obtained was depicted in Fig. 2.

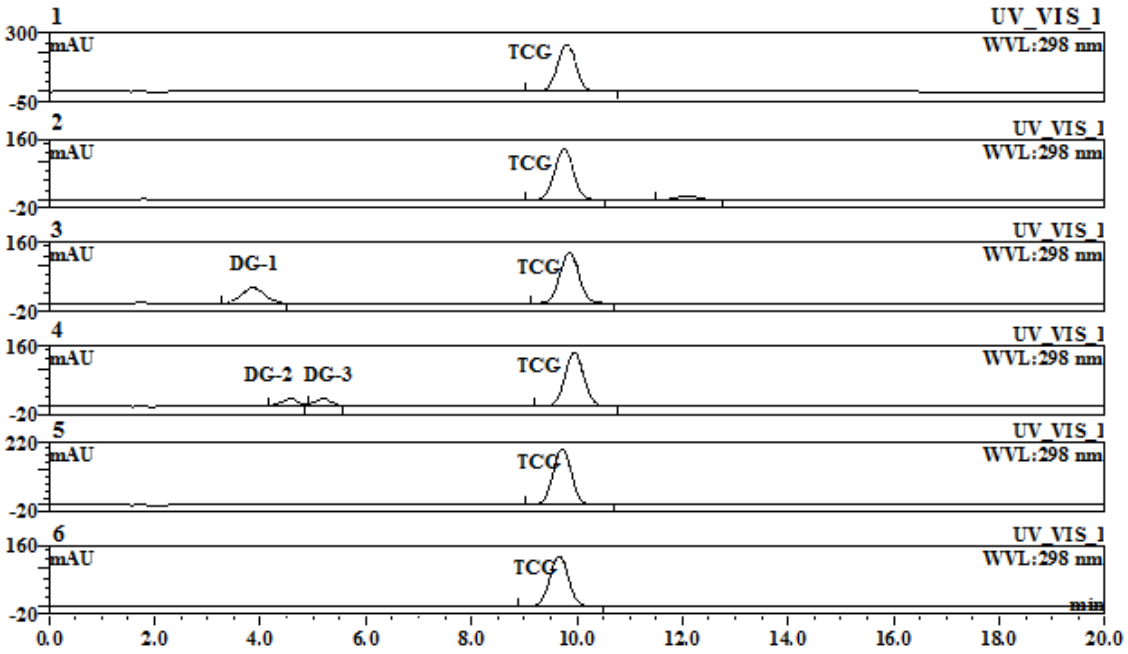

Fig. 2: HPLC-PDA chromatograms of (a) Ticagrelor in (b) acidic, (c) alkaline, (d) oxidative, (e) photolytic and (f) dry heat induced conditions

CONCLUSIONS

A simple and convenient HPLC-PDA method was developed and validated to perform the stability indicating study and quantification of TCG in Brilinta tablets. TCG was found to be stable under acidic, photolytic and thermolytic conditions and the significant degradation was observed under alkaline and oxidative conditions. As the results obtained were in good agreement with label claim developed method can be applied for regular analysis in quality control laboratory.

REFERENCES

1. Springthorpe B, Bailey A, Barton P, Birkinshaw TN, Bonnert RV and Brown RC. From ATP to AZD6140: The Discovery of an Orally Active Reversible P2Y12 Receptor Antagonist for the Prevention of Thrombosis. Bioorg Med Chem Lett. 2007;17: 6013-18.
2. Bonello L, Laine M, Kipson N, Mancini J, Helal O and Fromonot J. Ticagrelor increases adenosine plasma concentration in patients with an acute coronary Syndrome. J Am Coll Cardiol. 2014;63:872-7.
3. Berger JS. Aspirin, Clopidogrel, and Ticagrelor in Acute Coronary Syndromes. Am J Cardiol . 2013;112:737-45.
4. Schneider DJ, Agarwal Z, Seecheran N, Keating FK, Gogo P. Pharmacodynamic Effects during the Transition Between Cangrelor and Ticagrelor. J Am Coll Cardiol Intv. 2014;7:435-42.
5. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H and Held C. Ticagrelor versus Clopidogrel in Patients with Acute Coronary Syndromes. N Engl J Med. 2009;361:1045-57.
6. Henry D and Satgunam S. Idiopathic omental bleeding. J Sur Case Rep. 2012;9:2-3.
7. Cheng VE, Oppermen A, Natarajan D, Haikerwal D and Pereira J. Spontaneous Omental Bleeding in the Setting of Dual Anti-platelet Therapy with Ticagrelor. Heart, Lung and Circulation. 2014;23:e115-e117.
8. Sillen H, Cook M and Davis P. Determination of ticagrelor and two metabolites in plasma samples by liquid chromatography and mass spectrometry. J Chromatogr B. 2010;878:2299-306.
9. Sillen H, Cook M and Davis P. Determination of unbound ticagrelor and its active metabolite (AR-C124910XX) in human plasa by equilibrium dialysis and LC–MS/MS. J Chromatogr B. 2011;879:2315-22.

10. Kalyani L and Rao AL. A validated stability-indicating HPLC method for determination of ticagrelor in bulk and its formulation. Int J Pharm. 2013;3:634-42.
11. ICH-Q1A (R2) Validation of Analytical Procedure: Definitions and Terminology, Stability Testing of New Drug Substances and Products, Geneva 2003.
12. Tamaro I, Aprile S, Giovenzana GB and Grosa G. Development and validation of a stability-indicating HPLC-UV method for the determination of alizapride and its degradation products. J Pharm Biomed Anal. 2010;51:1024-31.
13. Marques LA, Maada I, de Kanter FJJ, Lingeman H, Irth H and Niessen WMA. Stability-indicating study of the anti-Alzheimer's drug galantamine hydrobromide. J Pharm Biomed Anal 2011;55:85-92.
14. ICH-Q2B Validation of Analytical Procedure: Text and methodology, International Conference of Harmonization, Geneva, 1996.