Research Article

Spectrophotometric methods for determination of ticagrelor in dosage forms

Effat Souri[1*], Kawthar Majid Hamid[1], Maliheh Barazandeh Tehrani[1], Hassan Jalalizadeh[2]

[1]*Department of Medicinal Chemistry, Faculty of Pharmacy and Drug Design and Development Research Center, Tehran University of Medical Sciences, Tehran (14155-6451), Iran*

[2]*Department of Research and Development, Alborz-Zagros Pharmaceutical Company, Tehran, Iran*

Received: 28 August 2017 Revised: 12 September 2017 Accepted: 28 September 2017

Abstract

Objective: The aim of this study was to develop simple and rapid spectrophotometric method for determination of ticagrelor in dosage forms. **Materials and methods:** Two rapid and accurate spectrophotometric methods were described for the determination of ticagrelor in pharmaceutical dosage forms. In the first method direct spectrophotometry was used for the quantification of ticagrelor at 255 nm. The second method was based on the ion-pair complex formation of ticagrelor and bromocresol green extractable in chloroform. After optimization of the reaction, the resulting complex was measured at 416 nm. **Results and conclusion:** Linear calibration curves were obtained at the range of 0.5-25 µg mL^{-1} and 5-1000 µg mL^{-1} for the direct and ion-pair complex formation method, respectively. Validation parameters were satisfactory for both methods. The proposed methods were applied for the determination of ticagrelor in tablets without any interference from excipients.

Keywords: Ticagrelor, Bromocresol green, Ion-pair complex formation, Spectrophotometry

Introduction

Ticagrelor (Figure 1) is a member of agents known as cyclopentyltriazolopyrimidine with platelet aggregation activity which is used for the prevention of thrombotic complications in patients with acute coronary syndrome (Husted and Giezen, 2009). Ticagrelor is a reversible and selective platelet p_2y_{12} receptor antagonist which inhibits the adenosine phosphate (ADP) induced platelet aggregation and thrombus formation (Husted et al., 2006). Ticagrelor is an orally active platelet p_2y_{12} receptor antagonist and showed rapid onset of action and enhanced effect compared with clopidogrel (Cannon et al., 2007; Gurbel et al., 2009; Husted et al, 2006).

LC/MS/MS method has been used before for the determination of ticagrelor and its metabolites in plasma (Sillen et al., 2010). Also, a stability-indicating HPLC method has been developed for the determination of ticagrelor in bulk powder and dosage forms (Kalyani and Lakshmana Rao, 2013).

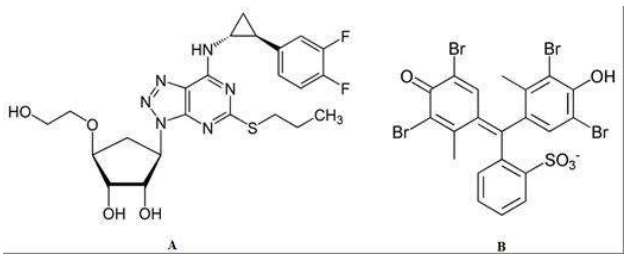

Figure 1. Chemical structure of ticagrelor (A) and BCG (B).

In the first method direct spectrophotometric method was used. The second method was based on the ion-pair complex formation of ticagrelor and bromocresol green (BCG) (Figure 1). Up to now, to the best of our knowledge, no spectrophotometric method is reported for the determination of ticagrelor based on its complex formation with bromocresol green. Bromocresol green formed a colored ion-pair complex with amino group which is extractable in organic solvents such as chloroform.

Materials and methods

Apparatus

Spectrophotometric measurements were made on a double

*Address for Corresponding Author:
Dr. Effat Souri
Department of Medicinal Chemistry,
Faculty of Pharmacy and Drug Design and Development Research Center, Tehran University of Medical Sciences, Tehran (14155-6451), Iran. Phone: (98 21) 66959065; Fax : (98 21) 66461178
E-mail: souri@sina.tums.ac.ir

beam UV-visible spectrophotometer (UV-160 Schimadzu, Japan). A Metrohm (Switzerland) pH meter was used for adjusting the pH.

Chemicals

Ticagrelor bulk powder (Hengtai, China, Batch No: 10600920140101) and Ticagrelor 90 mg tablets (Osvah Pharmaceutical Company, Tehran, Iran Experimental Batch No: 004) were kindly provided by Osvah Pharmaceutical Company. Bromocresol green (BCG) was purchased from Merck (Darmstadt, Germany). Other chemicals and analytical grade solvents were from Merck.

Standard solutions

Method A: A stock standard solution of ticagrelor was prepared in methanol (100 μg mL^{-1}). Calibration solutions of ticagrelor were prepared in methanol in the concentration range of 0.5-25 μg mL^{-1} (0.5, 1, 2, 5, 10, 15, 20, and 25 μg mL^{-1}).

Method B: An aqueous solution of bromocresol green (10×10^{-4} M) was prepared by dissolving appropriate amount of BCG in water in the presence of 0.2% of 0.1M sodium hydroxide. Stock standard solution of ticagrelor (10×10^{-4}) was prepared in methanol-water (50: 50, v/v). Phosphate buffer 0.1 M was prepared by dissolving 1.56 g of NaH_2PO_4 in 100 mL of water and adjusting the pH at 2.0 by adding 2M phosphoric acid solution.

Calibration solutions of ticagrelor were prepared in the concentration range of 5-1000 μg mL^{-} (5, 10, 20, 50, 100, 200, 500, and 1000 μg mL^{-1}) after appropriate dilution of a stock solution (2000 μg mL^{-1}) in methanol-water (50: 50, v/v).

Britton-Robinson buffer was prepared by mixing equal volumes of 0.1 M H_3PO_4, 0.1 M H_3BO_3, and 0.1 M CH_3COOH and adjusting the pH at 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0.

General procedure for sample preparation

Method A: The absorbance of standard or calibration solutions of ticagrelor in methanol was measured at 255 nm.

Method B: Two ml aliquots of ticagrelor solution were transferred into a 100 mL separating funnel. Five ml of the BCG reagent, 1 mL of buffer solution and 2 mL of water were added and mixed. The reaction mixture was extracted 3 times by chloroform using 5, 3, and 2 mL of the solvent. The chloroform layers were passed through anhydrous sodium sulfate and transferred into a 10 mL volumetric flask. The volumetric flask was made up to volume with chloroform. The absorbance of the yellow colored ion-pair complex was determined at 416 nm against chloroform as blank.

Optimization of the reaction conditions of method B

Selection of suitable pH: The effect of pH on ion-pair complex formation was studied by using the general procedure and Britton-Robinson buffers in the range of 1.5-4.0. Also different kinds of buffers (phthalate, acetate, phosphate, and Britton-Robinson) at the same pH value were studied to find out the effect of buffer type.

Selection of reagent amount*:* Using 2 mL of ticagrelor solution and 1 ml of phosphate buffer solution (pH 3.0), different amounts of BCG reagent (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, and 7 mL) were added and after extraction, the absorbance of the resulting ion-pair complex was measured at 416 nm.

Selection of the extracting solvent: Chloroform, dichloromethane, ethyl acetate and diethyl ether were used as extracting solvent and the absorbance of the resulting ion-pair complex was compared to find out the best extracting solvent.

The effect of reaction time: The effect of time on the ion-pair complex formation was studied at various time intervals in the range of 0-60 min.

Composition of the ion-pair complex: Job's method of continuous variation was employed to find out the stoichiometry of the ion-pair complex formation. The ticagrelor and BCG solutions at the same concentration level (10×10^{-4} M) were used to prepare a series of solutions with the total volume of 10 mL. The drug and reagent were mixed in various proportions (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, and 10:1 v/v) and determined as directed in the general procedure. The absorbance was measured at 416 nm.

Linearity

Method A: Six series of ticagrelor solutions in methanol in the concentration range of 0.5-25 μg mL^{-1} were determined at 255 nm. The calibration curves were constructed and statistical data calculated.

Method B: Six series of ticagrelor solutions in the range of 5-1000 μg mL^{-1} in methanol-water (50:50, v/v) were prepared. These solutions were treated according to the general procedure for method B. After construction of calibration curves, the statistical data were calculated.

Precision and accuracy

To find out the within-day accuracy and precision of the method, three different concentration levels of ticagrelor solutions (1, 10, and 25 μg mL^{-1} for method A and 10, 100, and 1000 μg mL^{-1} for method B) were assessed in triplicate using the general procedure. The between-day accuracy and precision were determined by repeating the same procedure for three consecutive days.

Determination of ticagrelor in dosage forms

Twenty Ticagrelor tablets were crushed into fine powder using a mortar and pestle. An amount of the powder equivalent to 30 mg of ticagrelor transferred in to a 100 mL volumetric flask. 70 mL of methanol were added. The mixture was sonicated for 20 min and made up to volume by the same solvent. A portion of the solution was filtered through a 0.45 µm Syringe filter (Teknokroma, Spain). The filtrate was determined according to the general procedure (Method A) after appropriate dilution. The absorbance was compared with the absorbance of a standard solution of ticagrelor at the same concentration value. The same treatment procedure was performed for the preparation of assay solution for Method B, by using a mixture of methanol and water (50:50, v/v) as solvent.

Relative recovery

Standard addition method was used to find out the relative recovery of the proposed methods. Standard solution of ticagrelor was added to an assay solution and the resulting mixture was determined according to the general procedure. The absorbance was compared with the absorbance of a standard solution of ticagrelor at the same concentration level to find out the relative recovery.

Statistical Analysis

All experiments were performed in triplicate and mean and standard deviation of the resulting data were reported.

Results

Absorption spectra

Method A: The absorption spectrum of ticagrelor in methanol showed a maximum absorbance at 255 nm which was used for spectrophotometric determinations.

Method B: The absorption spectra of formed ion-pair complex of ticagrelor and bromocresol green extracted with chloroform against a blank solution showed a maximum absorbance at 416 nm. This wavelength was used for spectrophotomertic measurements. No absorption was observed in the visible region for ticagrelor or bromocresol green solution alone.

Optimization of ion-pair complex formation

Selection of suitable pH: To find out the optimum pH of the buffer for complex formation Britton-Robinson buffer at different pH values in the range of 1.5-4.0 were used. The best pH value which shows higher absorption was equal to 2.0 (Figure 2). Higher or lower pH values caused a significant decrease in absorption value. It was also shown that better results were obtained by using a phosphate buffer at pH 2.0 instead of other kinds of buffers.

Selection of reagent amount:

Maximum absorption of formed ion-pair complex was observed by using 5 mL of the BCG reagent. Higher amounts of the reagent did not show any effect on the reaction yield (Figure 3).

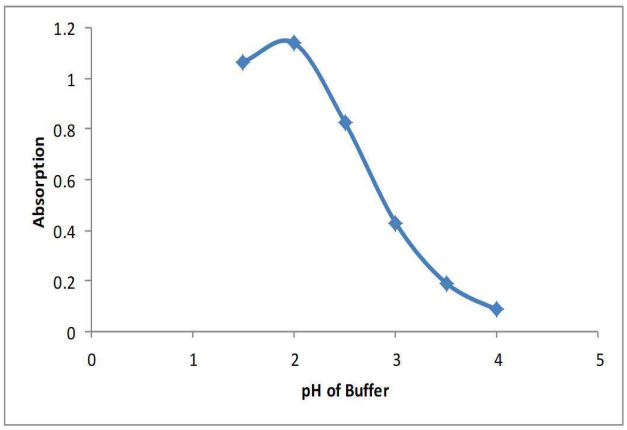

Figure 2. The effect of pH of the buffer (Britton-Rabinson) on the ion-pair complex formation

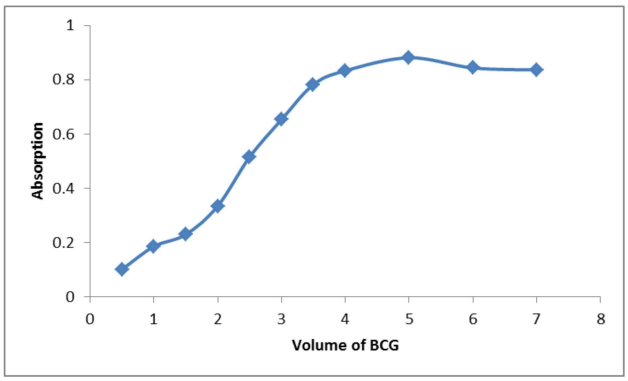

Figure 3. The effect of BCG amount on the absorbance of the ion pair complex at 416 nm.

Selection of the extracting solvent: By using several organic solvents such as chloroform, dichloromethane, ethyl acetate and diethyl ether for extraction of the ion-pair complex form aqueous phase, chloroform showed highest absorption which was used as the extraction solvent.

The effect of reaction time: The effect of time on the ion-pair complex formation was studied. Maximum absorbance was achieved immediately after mixing the ticagrelor and bromocresol green solution in the presence of buffer solution. It was also observed that addition of the buffer solution after premixing the drug and reagent gave better results.

Stability of the ion-pair complex: It was observed that the ticagrelor-bromocresol green ion-pair complex was relatively stable at room temperature at least for 1 h (recovery>99.5%). The ion-pair complex was relatively stable up to 24 h at room temperature (recovery>92%).

Composition of the ion-pair complex: Using the Job's continuous variation method, the stoichiometric of the formed ion-pair complex was shown to be at a 1:1 ratio (Figure 4).

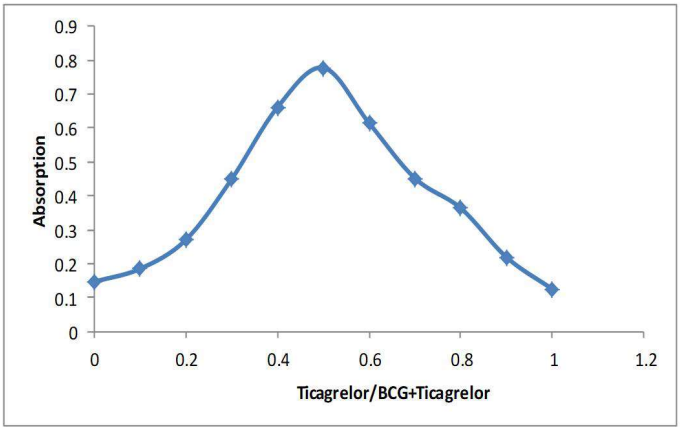

Figure 4. Stoichiometry of the ion pair complex of ticagrelor ($10x10^{-4}$ M) and BCG ($10x10^{-4}$ M) by Job's continuous variation method.

Method validation

Linearity: A linear relationship was observed between the absorption of ticagrelor and also ion-pair complex and ticagrelor concentration in the range of 0.5-25 µg mL^{-1} and 5-1000 µg mL^{-1}, respectively. The regression equation and statistical data for six calibration curves are shown in table 1.

Table 1. Statistical data of calibration curves of ticagrelor in standard solutions (n = 6)

Parameters	Method A	Method B
Linearity range	0.5-25 µg mL^{-1}	5-1000 µg mL^{-1}
Regression equation	y = 0.077 x + 0.027	y = 0.0009x + 0.049
Standard deviation of slope	0.0003	4.1×10^{-5}
Relative standard deviation of slope (%)	0.39	4.56
Standard deviation of intercept	0.006	0.005
Correlation coefficient (r^2)	0.9995	0.9988

Precision and accuracy: The within-day and between-day accuracy and precision of the proposed methods were investigated using three different levels of ticagrelor concentration. The results are shown in table 2. These data showed good and adequate accuracy and precision of the method for analytical purposes.

Relative recovery: The relative recovery of ticagrelor from a previously analyzed tablet solution after standard addition method was obtained to be 99.02±0.65% and 98.94±0.35% for method A and Method B, respectively.

Application of the method

The proposed methods were used for determination of ticagrelor in tablets. The results compared with the in-house HPLC method (Osvah Pharmaceutical Company) is shown in table 3. No significant difference was observed between the assay results of the proposed methods and reference HPLC method.

Table 2. Precision and accuracy of the method for determination of ticagrelor in standard solutions (n=9; 3 sets for 3 days)

Concentration added (µg mL^{-1})	Within-day (n=3)			Between-day (n=9)		
	Found (µg mL^{-1})	CV (%)	Error (%)	Found (µg mL^{-1})	CV (%)	Error (%)
Method A						
1.00	1.01±0.02	**1.98**	1.00	1.01±0.02	1.98	1.00
10.00	10.16±0.01	**0.10**	1.60	10.07±0.12	1.18	0.70
25.00	25.02±0.19	**0.76**	0.08	25.10±0.15	0.60	0.40
Method B						
10.00	9.96±0.10	1.00	-0.40	9.98±0.14	1.40	-0.20
100.00	98.97±1.10	1.11	-1.03	98.67±1.51	1.51	-0.33
1000.00	997.53±11.84	1.19	-0.25	1000.01±10.69	1.07	0.00

Table 3. Comparison of the developed methods with the reference method for the determination of Ticagrelor tablets

Method	Label claimed (mg)	Found (mean±sd*)
Method A	90.00	89.93±0.56
Method B	90.00	89.79±0.66
Reference HPLC method	90.00	89.89±0.19

*Standard deviation

Discussion

As mentioned before few HPLC method has been reported for determination of ticagrelor. Although HPLC methods show high degree of specificity, there is a need for high purity organic solvents and also these methods are more time consuming than spectrophotometric methods. Spectrophotometric methods are considered to be more convenient techniques because of their economical (low cost) and simplicity advantage over other instrumental techniques. Our target was to develop and validate two simple spectrophotometric methods for the determination of ticagrelor in bulk powder and pharmaceutical dosage forms. In the first method direct spectrophotometric method was used. In the second method bromocresol green was used as an ion-pair complexing reagent. Based on our goal for developing simple and rapid spectrophotometric methods for determination of active ingredients in pharmaceutical dosage forms, bromocresol green has been used for some other drugs before (Amanlou et al., 2007a; Amanlou et al., 2007b; Amanlou et al., 2007c; Amanlou et al. 2014; Souri et al., 2013). The formed ion-pair complex between ticagrelor and bromocresol green which is formed in acidic medium was extractable in chloroform and showed an absorbance at 416 nm. The complex formation

conditions were also optimized and the accuracy and precision were checked. Both methods showed suitable precision and accuracy for determination of active ingredient in pharmaceutical dosage forms. The proposed methods do not need any special pretreatment processes and this is one of the advantaged of these methods. Also these methods are very simple, accurate and cost effective.

Conclusion

The proposed methods are simple, rapid, and accurate for the determination of ticagrelor in bulk powder and tablet dosage form. No interferences from tablet excipients were observed. As the proposed methods are rapid and simple, they could be used as a routine analytical method for quality control assay of ticagrelor in pharmaceutical dosage forms.

Conflict of interest

The authors declare that there is no conflict of interests.

Acknowledgement

The authors would like to thank Ms Fereshteh Keyghobadi for her valuable contribution in experimental tasks.

References

Amanlou M, Khosraian P, Souri E, Ghorban Dadrass O, Dinarvand R, Alimord MM, Akbari H. 2007a. Determination of buprenorphine in raw material and pharmaceutical products using ion-pair formation. Bulletin of Korean Chemical Society, 28:183-187.

Amanlou M, Hoseinzadeh Nazlou, Azizan H, Souri E, Farsam H. 2007b. Determination of ketotifen fumarate in raw material and pharmaceutical products using ion-pair formation. Analytical Letters, 40:3267-3279.

Amanlou M, Souri E, Izady S, Farsam H. 2007c. Spectrophotometric determination of tropicamide in bulk and pharmaceutical formulations. Iranian Journal of Pharmaceutical Sciences, 3:43-50.

Amanlou M, Ghazi Moghadam A, Barazandeh Tehrani M, Souri E. 2014. Validated spectrophotometric method for determination of tamsulosim in bulk and pharmaceutical dosage forms. Iranian Journal of Pharmaceutical Research, 13:81-86.

Cannon CP, Husted S, Harrington RA, Scirica BM, Emanuelsson H, Peters G, Storey RF. 2007. Safety, tolerability, and initial efficacy of AZD6140, the first reversible oral adenosine diphosphate receptor antagonist, compared with clopidogrel, in patients with non-ST-segment elevation acute coronary syndrome: primary results of the DISPERSE-2 trial. Journal of the American College of Cardiology, 50:1844-1851.

Gurbel PA, Bliden KP, Bulter K, Tantry US, Gesheff T, Wei C, Teng R, Antonino MJ, Patil SB, Karunakaran A, Kereiakes DJ, Parris C, Purdy D, Wilson V, Ledley GS, Storey RF. 2009. Randomized double-blind assessment of the ONSET and OFFSET of the antiplatelet effects of ticagrelor versus clopidogrel in patients with stable coronary artery disease: the ONSET/OFFSET study. Circulation, 120:2577-2585.

Husted S, Giezen J. 2009. Ticagrelor: the first reversibly binding p_2y_{12} receptor antagonist. Cardiovascular Therapeutics, 27:259-274.

Husted S, Emanuelsson H, Heptinstall S, Sandset PM, Wickens M, Peters G. 2006. Pharmacodynamics, pharmacokinetics, and safety of the oral reversible p_2y_{12} antagonist AZD6140 with aspirin in patients with atherosclerosis: a double-blind comparison to clopidogrel with aspirin. European Heart Journal, 27:1038-1047.

Kalyani L, Lakshmana Rao A. 2013. A validated stability-indicating HPLC method for determination of ticagrelor in bulk and its formulations. International Journal of Pharmaceutics, 3(3):634-642.

Sillen H, Cook M, Davis P. 2010. Determination of ticagrelor and two metabolites in plasma samples by liquid chromatography and mass spectrometry. Journal of Chromatography B, 878:2299-2306.

Souri E, Kaboodari A, Adib N, Amanlou M. 2013. A new extractive spectrophotometric method for determination of rizatriptan dosage form using bromocresol green. DARU Journal of Pharmaceutical Sciences, 21:12.