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Evaluation of Protein Binding Based *In-Vitro* Interaction of Ticagrelor and Pantoprazole Using RP-HPLC

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ABSTRACT

Evaluation of protein binding based *in vitro* interaction between ticagrelor and pantoprazole was carried out by a reversed phase HPLC method. An efficient RP-HPLC method was developed for the simultaneous estimation of ticagrelor and pantoprazole and applied to evaluate protein binding based interaction of two drugs using equilibrium dialysis method. The amount of free and bound drugs was estimated by using reverse phase chromatographic method with photo diode array detection. Shimadzu C_{18} column (250 mm × 4 mm, 5 µ) was used to separate ticagrelor from pantoprazole. The mobile phase used was 10 mM potassium dihydrogen orthophosphate: methanol (20:80 %v/v) at a flow rate of 1 ml/min. The drugs were detected at wavelength of 254 nm and peak areas were integrated. The retention time for pantoprazole was 3.1 min and ticagrelor was 7.8 min. The developed method was validated and successfully applied for the evaluation of protein binding based *in-vitro* interaction of ticagrelor and pantoprazole. The protein binding study results have shown that there is a significant change in the binding of two drugs in presence of each other and hence might affect the effectiveness of them and lead to adverse effects.

Keywords: Ticagrelor, Pantoprazole, Protein binding, RPHPLC.

INTRODUCTION

Bioavailability of drugs are determined by their protein binding nature. When drugs having high binding nature are co-administered the protein binding based interactions are occurring. The competitive protein binding drug-drug interaction results in increased free plasma concentration of the displaced medications. A drug in plasma binds, much or less to plasma proteins such as albumin, α_1-acid glycoprotein

and in rare case with immunoglobulin and quickly establishes binding equilibrium. The plasma protein binding of drugs has been shown to have significant effects on numerous aspects of pharmacokinetics such as hepatic metabolism rate, renal excretion, bio membrane permeation rate and steady state distribution rate and pharmacodynamics [1].

These kinds of interactions are clinically significant for potent drugs. Hence it is need of the hour to develop effective analytical methodology to evaluate such interaction. An analytical method must be efficient enough to separate analyte of interest without interference of matrix and should enable quantification of them. Chromatographic separation techniques are very efficient of which HPLC offers high throughput analysis and greater sensitivity is the preferable method in drug development and research.

Ticagrelor is (1 S,2 S,3 R,5 S)-3-(7-(((1 R,2 S)-2-(3,4-difluorophenyl) cyclopropyl) amino)-5-(propyl sulfanyl)-3 H-(1,2,3) triazolo (4,5-d) pyrimidin-3-yl)-5-(2 hydroxyethoxy) cyclopentane-1,2-diol, chemically. It is antiplatelet drug used in acute coronary syndrome. It inhibits prothrombic effect of ADP by blocking platelet P_2Y_{12} receptor and the effect is reversible. It is available in tablet dosage forms. Pantoprazole is 6-(difluoromethoxy)-2-((3, 4-dimethoxypyridin-2-yl) methylsulfinyl)-1 H-1, 3-benzodiazole, chemically [2]. It is a proton pump inhibitor used in the treatment of gastroesophageal reflux disease, Zollinger-Ellison syndrome. Tablets and injections containing pantoprazole are frequently used. The protein binding based interactions of these drugs are inevitable when co-administered, since both drugs are reported to have protein binding more than 99%. Few analytical and bio analytical methods were reported for ticagrelor [3-9] and pantaprazole [10-13], for their estimation from individual dosage form or with other drugs. However, no study reported for the simultaneous estimation of two drugs for protein binding evaluation. The objective of this current work is to report an efficient RP-HPLC method for the simultaneous estimation of Ticagrelor and pantoprazole and to evaluate protein binding based interaction of two drugs using equilibrium dialysis method.

MATERIALS AND METHODS

Chemicals and reagents

Ticagrelor was purchased from clear synth, India with certificate of analysis. All the chemicals and reagents used were of AR grade and all the solvents were of HPLC grade.

Instrumentation

Shimadzu LC 2010 AD with PDA detector and LC-MS solution software was employed. Shimadzu C_{18} column (250 mm × 4 mm, 5 µ) was used to separate ticagrelor from pantoprazole.

Chromatographic conditions

The separation was carried out using 10 m M potassium dihydrogen orthophosphate: methanol (20:80 %v/v) at a flow rate of 1 ml/min and the detection wavelength at 254 nm. The sample injection volume was 20 µ L.

Preparation of drug solutions

A quantity of 5 mg of ticagrelor was weighed and dissolved in sufficient volume of DMSO. It was made up to 10 ml with methanol to obtain 500 µ g/ml. A working standard solution of 10 µ g/ml of ticagrelor was prepared with methanol. A quantity of 10 mg of pantoprazole was weighed and dissolved in methanol and make up to 10 ml with methanol to obtain 1000 µ g/ml. A working standard solution of 7 µ/ml of pantoprazole were prepared with methanol.

Separation of ticagrelor and pantoprazole by RP-HPLC: Mobile phase optimization was carried out in order to resolute

pantoprazole and ticagrelor with their ideal peak characters. The mobile phase system consisting of 10 mM potassium dihydrogen orthophosphate buffer and methanol was optimized by changing the proposition and PH of the buffer. The developed RP-HPLC method was validated in terms of specificity, linearity, accuracy, precision, robustness, LOD and LOQ as per ICH guidelines [14].

Evaluation of protein binding based interaction of ticagrelor and pantoprazole: The developed RP-HPLC method was applied for the *in-vitro* displacement interaction study between Ticagrelor and Pantoprazole. The *in-vitro* interaction study was carried out by equilibrium dialysis using a dialysis membrane. The Bovine Serum Albumin (BSA) solution was employed for the estimation of protein binding of the drugs. To prepare 2×10^{-5} M BSA solution 1.32 g of BSA was dissolved in HPLC grade water and made up to 100 ml. The dialysis membrane tubes were cut into each 15 cm length and its activation had been done by boiling the membrane for 2 hrs in 250 ml of HPLC grade water at 70°C (± 5°C). The boiled membranes were washed thoroughly with fresh HPLC grade water and utilized for the study.

The protein binding of the drug was estimated by measuring the unbound fraction of the drug. A concentration 2×10^{-5} M of drug solutions and BSA were prepared. The solutions were taken in the activated dialysis membrane tube and sealed and they were immersed in buffer contained in the measuring cylinders placed in a mechanical shaker at 40 rpm for sufficient period of time to complete dialysis. The samples withdrawn from the buffer compartment were analysed using the newly developed HPLC technique. The chromatograms were recorded and the concentration of unbound drug was determined by calculating peak area ratios with the standards. The percentage of protein binding (F) was calculated using the formula F=(B-A)/B × 100, where, A=Concentration of free drug in buffer compartment, B=Concentration of total drug in buffer compartment. The difference in the percentage protein binding (F) of Ticagrelor, with and without pantoprazole was calculated to find the displacement of Ticagrelor due to interaction.

RESULTS AND DISCUSSION

An RP-HPLC method was developed for the separation of ticagrelor and pantoprazole in admixture by optimizing the chromatographic conditions and it was validated. The drugs were retained at 3.1 min (pantoprazole) and 7.8 min (ticagrelor), and total analysis time was 10 min. A good linearity was observed in the concentration of 1.5-7.5 µ g/ml and 0.5-2.5 µ g/ml respectively, for ticagrelor and pantoprazole. The calibration graph was plotted with measured peak areas of the drug against concentrations. The peak area of these solutions was measured at 254 nm respectively. The calibration graph for both the drugs is shown in Figures 1 and 2. The correlation coefficient values were found to be >0.99 for both the drugs.

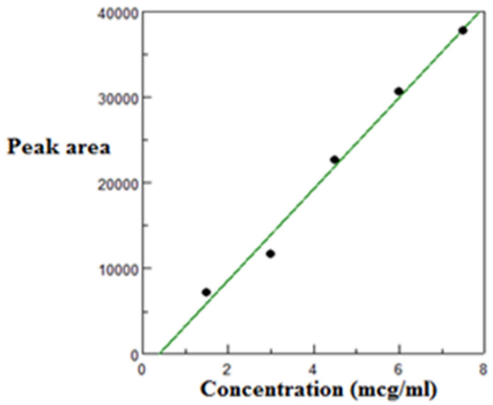

Figure 1: Calibration graph of ticagrelor.

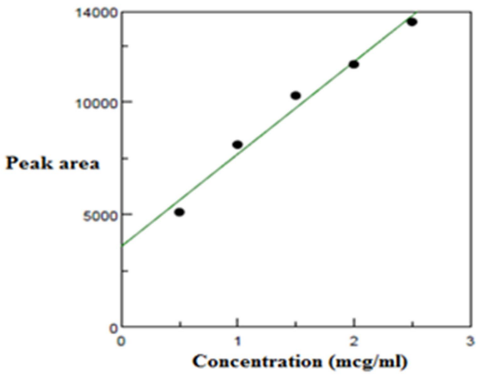

Figure 2: Calibration graph of pantoprazole.

The precision was determined in terms of intraday precision, inter-day precision and repeatability by injecting solutions of Ticagrelor and Pantoprazole in linearity range of two drugs %RSD was calculated. The LOD was found to be 0.2 µg/ml for Ticagrelor and 0.5 µg/ml for Pantoprazole. The LOQ was found to be 1.5 µg/ml for Ticagrelor and 0.5 µg/ml for Pantoprazole.

There were no additional peaks observed while injecting solvents or mobile phase alone. The peak purity index of standard Ticagrelor and Pantoprazole were found to be 0.9999 for both the drugs which proves specificity of the method. The stability of drug solutions was recorded at room temperature and at refrigerated conditions. The solutions were found to be stable for 12 h. The system suitability parameters like peak area, tailing factor, and theoretical plate count, resolution and retention time were calculated from standard chromatograms. The system suitability parameter data for Ticagrelor and Pantoprazole is shown in Table 1.

Drug	Theoretical plate (N)	Retention time (min)	Tailing factor (Tf)	Resolution
Ticagrelor	2112.269	8.7	1.3	
Pantoprazole	1185.569	3.2	0.9	8.5

Table 1: System suitability parameters for ticagrelor and pantoprazole.

Application of RP-HPLC method for protein binding based *in-vitro* interaction of Ticagrelor and Pantoprazole. The Percentage protein binding of Ticagrelor and Pantoprazole was estimated by equilibrium dialysis method. The various concentrations of Ticagrelor and Pantoprazole were prepared and their percentage protein binding was determined. The percentage protein binding of individual Ticagrelor and pantoprazole after 2 h was found to be 92.4 and 83.78 respectively.

The chromatogram of ticagrelor and pantoprazole after equilibrium dialysis with BSA shown in Figure 2. Results of *in-vitro* interaction study of ticagrelor and pantoprazole are shown in Table 2. The *in-vitro* displacement interaction was carried out for ticagrelor in the presence of its interacting drug Pantoprazole.

The results of *in-vitro* drug interaction study between ticagrelor and pantoprazole show that percentage protein binding of pantoprazole in presence of ticagrelor was reduced, thereby the percentage displacement of pantoprazole in presence of ticagrelor increases, which can lead to anaphylaxis and other severe hypersensitivity reactions. Immediate medical intervention of drug discontinuance is required if anaphylaxis of hypersensitivity reaction occurs. Increased concentration of pantoprazole may cause acute renal failure and abnormal liver function suggesting hepatitis. Hence monitoring is essential when pantoprazole is co-administered with ticagrelor (Figure 3 and Table 2).

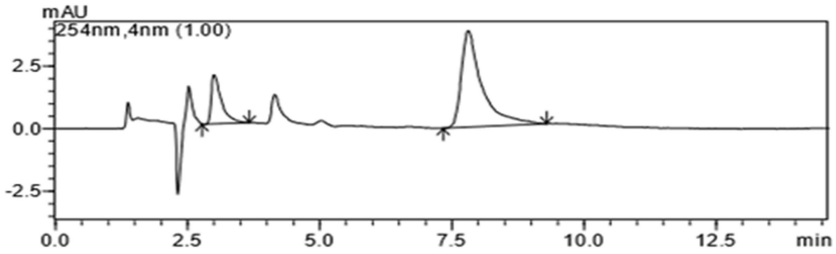

Figure 3: Chromatogram of ticagrelor and pantoprazole after equilibrium dialysis.

Concentration (µ g)		Concentration of unbound drug (µ g)		% Protein binding		% Displacement *	
T	P	T	P	T	P	T	P
10	7	0	4.04	100	57.5	100	26.38
Note: *An average of 6 observations: T- Ticagrelor and P-Pantoprazole							

Table 2: Results of *in-vitro* interaction study of ticagrelor with pantoprazole.

CONCLUSION

A RP-HPLC method was developed for ticagrelor and its co-administered drug pantoprazole. The method was validated and applied for evaluation of protein binding study. The *in-vitro* evaluation of protein binding was done by equilibrium dialysis method using BSA for two hours. The two drugs were well resolved with RS >2.

The protein binding of individual pantoprazole and ticagrelor at end of two hours was 83.78% and 92.45%, respectively. While they were evaluated in presence of each other, the percentage protein binding was found as 57.50% for pantoprazole and 100% for ticagrelor at the end of the study. The results obtained from the *in-vitro* interaction studies indicate that the drugs should be judiciously chosen for the concomitant therapy. Ticagrelor should be avoided in patients with severe hepatic impairment as the drug is primarily metabolized by liver.

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