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Biorelevant dissolution studies of platelet aggregation inhibitor: Ticagrelor hydrochloride and its co-crystal with sodium salt of Aspirin

M A Ambasana*[,1], N P Kapuriya[1], P A Ambasana[2], J J Bhalodia[1] & A H Bapodra[1]

[1]Department of Chemistry and Forensic Science, Bhakta Kavi Narsinh Mehta University, Junagadh, Gujarat, India

[2]Department of Chemistry, Shree M. & N. Virani Science College, Rajkot, Gujarat, India

E-mail: ambasanamrunal@gmail.com

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The comparative dissolution characteristics of poorly soluble platelet aggregation inhibitor Ticagrelor hydrochloride and its co-crystal with sodium salt of Aspirin in biorelevant media have been demonstrated. The API and co-crystal both are subjected to simulated gastric fluid (SGF) and fasted intestinal fluid without micelle forming components (blank FASSIF). The release of API is online monitored through reverse phase liquid chromatography. Prior to online monitoring, the chromatographic method is statistically validated in accordance with ICH guidelines. Chromatographic data reveals that the overall release of Ticagrelor after 3 h is about 6µg/mL higher in co-crystal (24.5µg/mL) compared to unaccompanied API (18.6µg/mL) in simulated gastric fluid (SGF). Whereas in fasted intestinal fluid without micelle forming components (blank FASSIF) more than threefold high release of API is observed in co-crystal (12.14µg/mL) compared to API (4.22µg/mL) in free form. Results clearly indicate the improved solubility in the lower regions of the gastrointestinal tract and better absorption of drug. This type of co-crystal would also allow the simultaneous dosing in suitable formulation.

Keywords: Biorelevant, Co-crystal, Dissolution, HPLC, Ticagrelor

In pharmaceutical science bioavailability is considered as one of the challenging parts for effectiveness of the drug. Many times, the most promising molecules fail to deliver desirable results due to less bioavailability or poor solubility in gastric fluids. Polymorphism, salts, and hydrates have been used since long for improvement of solubility. In recent times the co-crystallization approach has emerged as simple and promising way for improved solubility and bioavailability via preparation of novel co-crystals of active pharmaceutical ingredients[1,2].

The dissolution studies of APIs play active role in defining dissolution test specifications. It may also be used to confirm the persistent equivalence of the product, as well as the product's monotony after scale-up. The detailed and robust method for dissolution studies of APIs comprised a base for dissolution test specification and provides guideline for formulation and development.

The use of biorelevant media in dissolution studies of API has become widespread in the pharmaceutical research for better understanding that how drugs and formulations behave in the gastrointestinal tract[3]. These media aim to simulate the composition of the gastrointestinal contents more closely than the media that are commonly used in dissolution studies for routine quality control testing[4,5]. Until now, however, very less attempts have been reported for biorelevant dissolution studies of Ticagrelor and its co-crystals.

Ticagrelor hydrochloride is white to off white amorphous or crystalline solid. It belongs to the biopharmaceutical classification system (BCS) class IV molecules[6,7]. It is poorly soluble in water and sparingly soluble in methanol and in ethanol[7-8]. It does not exhibit *p*H-dependent solubility and no pKa value within the physiological range[8]. Ticagrelor is orally active, reversibly binding P2Y12 antagonist inhibiting platelet aggregation via P2Y12 ADP receptor[9,10]. Ticagrelor lowers the thrombotic cardiovascular risks in patients suffering from acute coronary syndrome. Ticagrelor and its most metabolite reversibly interact with the P2Y12 ADP-receptor to lower the platelet aggregation and thrombus formation[11-13].

The selection of Aspirin as co-former has always been a first choice for preparation of co-crystals due to high aqueous solubility and wide functionality in different medicaments. It is often used as a model or reference compound in pharmaceutical research[14]. Aspirin is a non-steroidal anti-inflammatory drug (NSAID) and works similarly to other NSAIDs. Moreover, it suppresses the normal functioning of platelets and hence used for the prevention of heart attacks, strokes, and blood clots in people at high risk[15]. Sodium salt of Aspirin has also been utilized since very long due to its enhanced solubility characteristics[16].

Literature survey reveals a US patent claiming that micro dissolution data in aqueous buffers at physiologically relevant *p*H demonstrate that

Ticagrelor Aspirin co-crystal has better solubility in non-micellar systems compared Ticagrelor hydrochloride[17]. It means the co-crystal is expected to have improved solubility in the lower regions of the gastrointestinal tract, which may result in improved absorption of Ticagrelor co-crystal from this region when taken as a co-crystal rather than Ticagrelor in the free form[18].

This would increase the possibility of achieving a modified release formulation to deliver Ticagrelor over an extended period, for example 12-24 h that could provide suitable plasma exposures and daily single dosing. Furthermore, the co-crystal of Ticagrelor hydrochloride with sodium salt of Aspirin would also permit the simultaneous dosing of both Ticagrelor and Aspirin[19].

Experimental Section

Materials and Reagents

The required quantities of samples and reference standards were provided by ANLON CRO, Rajkot (Gujarat) India. Analytical grade solvents, acids, salts and required organic compounds acid were procured from Merck India Limited (Mumbai, India). The 0.45micron PTFE membrane disc filters were obtained from Pall Corporation (Mumbai, India). High purity deionized water was obtained from Millipore, Milli-Q water purification system (Milford, MA, USA).

Apparatus

The shimadzu ATX 124 (repeatability SD $\leq$ 0.1 mg) analytical balance was used throughout the studies for weighing. The dissolution analysis was performed using Labindia DL-8, rotating paddle type USP 2 apparatus at paddle speed of 50 rpm, 37°C ± 0.2°C temperature and 250 mL volume of media.

Preparation of co-crystal

Accurately weigh and transfer 500 mg Ticagrelor (0.96 mmol) and 240 mg sodium salt of Aspirin (1.25 equivalent) to a flask, containing 7 mL ethyl acetate. The reaction mixture was stirred with quick heating and fast cooling for 2 h to get a clear solution. Remove ethyl acetate under reduced pressure and add about 14 mL n-heptane to get solid product. Filter the solid and wash with *n*-Heptane to obtained co-crystal[17].

Preparation of dissolution media

Simulated gastric fluid (SGF)

Simulated gastric fluid (SGF) was prepared by adding 131.5 mL 1M HCl to 4.0 g NaCl and the resulting solution made up to 2 L with deionized water.

Blank fasted state simulating intestinal fluid (FASSIF)

Blank fasted state simulating intestinal fluid (without micelle forming components) was prepared from 0.35 g NaOH pellets, 3.95 g $NaH_2PO_4.H_2O$ and 6.18 g NaCl in 1 L deionized water. The *p*H was then adjusted to *p*H 6.5 with 1N NaOH or 1N HCl.

Sampling points

3 mL of aliquot was taken at 00, 10, 20, 30, 60, 90 and 180 min, filtered through 0.45 micron PTFE membrane disc filter and analyzed by HPLC. The sample volume was replaced with an equal volume of fresh dissolution medium.

In-vitro dissolution studies

In-vitro dissolution studies were performed by transferring 10 mg of Ticagrelor hydrochloride and 10 mg of Ticagrelor co-crystal with sodium salt of Aspirin both in 250 mL of (i) simulated gastric fluid (SGF) and (ii) fasted state simulating intestinal fluid without micelle forming components (blank FASSIF). The paddle speed maintained at 50 rpm and temperature at 37°C ± 0.2°C. 3 mL of aliquot was taken at appropriate time intervals, filtered through 0.45 micron PTFE membrane disc filter and analyzed by HPLC.

Analytical method

Instrumentation

The chromatographic analysis was performed using Agilent Infinity 1220, Infinity Fast-LC (Pressure limit up to 600 bars) with auto sampler and PDA detector. Data acquisition and data processing was evaluated with Open Lab Chem Station.

Chromatographic condition

Online chromatographic monitoring was executed on ZORBAX Eclipse Plus 300SB C_{18} (250×4.6mm, 5.0µ) column. Mobile phase consists of (A) Acetonitrile and (B) 20mM Potassium dihydrogen ortho phosphate buffer (A: B,40:60 v/v) at a flow rate of 1.0 mL/min. The mobile phase was filtered through 0.45 micron PTFE disc filter before use. The eluent was monitored using PDA detector at wavelength 225 nm. The column was maintained at ambient temperature and the injection volume was 5 µL.

Preparation of Ticagrelor hydrochloride standard solution

Standard solution (227µg/mL) was prepared by transferring 9.08 mg of Ticagrelor hydrochloride into a 25 mL volumetric flask and adding about 20 mL

methanol. The solution was sonicated for 2-3 min to dissolve the sample and the solution was then diluted to volume with the same solvent. These solutions were utilized as standard preparation during online dissolution monitoring of API in (i) SGF and (ii) FASSIF.

Preparation of Co-crystal standard solution

Standard solution (382 µg/mL) was prepared by transferring 9.56 mg of Ticagrelor hydrochloride co-crystal with sodium salt of Aspirin into a 25 mL volumetric flask and adding about 20 mL methanol. The solution was sonicated for 2-3 min to dissolve the sample and the solution was then diluted to volume with the same solvent. These solutions were utilized as standard preparations during online dissolution monitoring of co-crystal in (i) SGF and (ii) FASSIF.

Method validation

Prior to analyzing dissolution aliquots, the liquid chromatographic method was statistically validated for accuracy, precision and linearity as per ICH Guidelines[20]. The limit of detection and limit of quantification were found 0.05 µg/mL and 0.20 µg/mL respectively hence the method is suitable for the estimation of Ticagrelor up to trace level (Table 1).

Result and Discussion

In this work, comparative biorelevant dissolution studies were carried out for Ticagrelor hydrochloride and its co-crystal with sodium salt of Aspirin. Simulated gastric fluid (SGF) and fasted intestinal fluid without micelle forming components (blank FASSIF) were employed as biorelevant media and solubility of both materials in these media were online monitored by HPLC. Prior to dissolution studies the analytical method was developed and statistically validated in accordance ICH guidelines.[20]

The basic chromatographic conditions were selected after testing the different conditions that affect HPLC analysis, for example column, aqueous and organic components of the mobile phase, ratio of mobile phases, detector wavelength, diluents and concentration of analyte. The ZORBAX Eclipse Plus 300SB C_{18} column was selected based on high resolving capacity, better reproducibility, low-back pressure, and low tailing. For mobile phase selection, preliminary trials using mobile phases of different composition containing water adjusted to acid *p*H by addition of o-phosphoric acid and methanol resulted in poor peak shape. When methanol was replaced by acetonitrile better peak shape was obtained (Fig. 1). The ratio of mobile phase was optimized to reduce retention times and enable good resolution of Ticagrelor hydrochloride from Aspirin (Fig. 2a). The detection wavelength of 225 nm was preferred after scanning the standard preparation over the UV range.

Selection of right dissolution conditions is the first and foremost step to characterize the solubility of the API. Simulated gastric fluid (SGF) and fasted intestinal fluid without micelle forming components (blank FASSIF) were selected as the main media for investigation as currently these fluids are most often utilized in pharmaceutical research and development[3].

In the first part of dissolution experiment, known amount of API and co-crystal were added to two separate vessels containing 250 mL of simulated

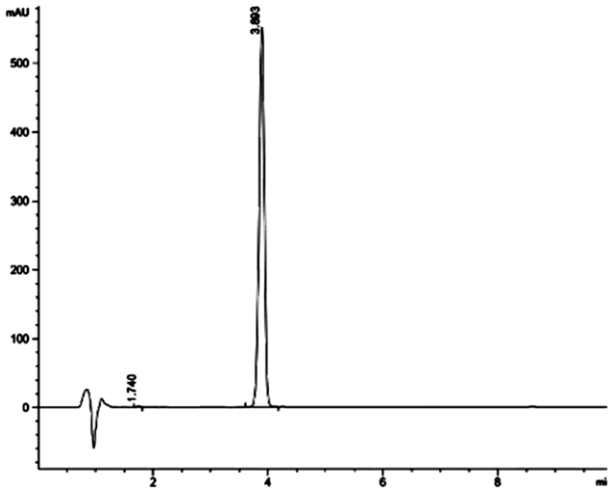

Fig. 1 — Chromatogram of Ticagrelor hydrochloride standard preparation

Table 1 — Method validation

Parameters	System suitability			Result
	RSD%	Theoretical plates	Asymmetry	
In house limit	NMT 2.0	NLT 8000	NMT2.0	
Recovery	0.45	8871	1.03	99.6 - 100.3%
Method Precision	0.32	8606	1.04	0.51%
Intermediate Precision	0.38	8413	1.04	0.53%
Linearity	0.66	8960	0.97	0.9989

gastric fluid (SGF). Both the solutions were maintained at 37°C ± 0.2°C temperature and rotated gently at paddle speed of 50 rpm.

3 mL of aliquots were taken at 10, 20, 30, 60, 90 and 180 min from both the preparation, filtered through 0.45micron PTFE membrane disc filter and analyzed by HPLC. The percentages of API dissolved in simulated gastric fluid (SGF) were determined by comparing the area of sample against standard preparation. Chromatographic data reveals that the overall release of API was about 4 to 6 ppm higher for co-crystal compare to alone API in entire time line (Table 2, Fig. 3a).

In second half, the entire experiment was re-performed by taking the fasted intestinal fluid without micelle forming components (blank FASSIF) in place of simulated gastric fluid (SGF). There was no release of API up 10 min, about 2.03 ppm release after 1 h and about 4.22 ppm release after 3 h, whereas when co-crystal added to FASSIF, 1.84 ppm API observed after 10 min, 9.25 ppm after 1 h and 12.14 ppm after 3 h. The results obtained by liquid chromatographic monitoring clearly indicates the better solubility characteristics of API with sodium salt of Aspirin co-crystal compare to alone API (Table 3, Fig. 2b).

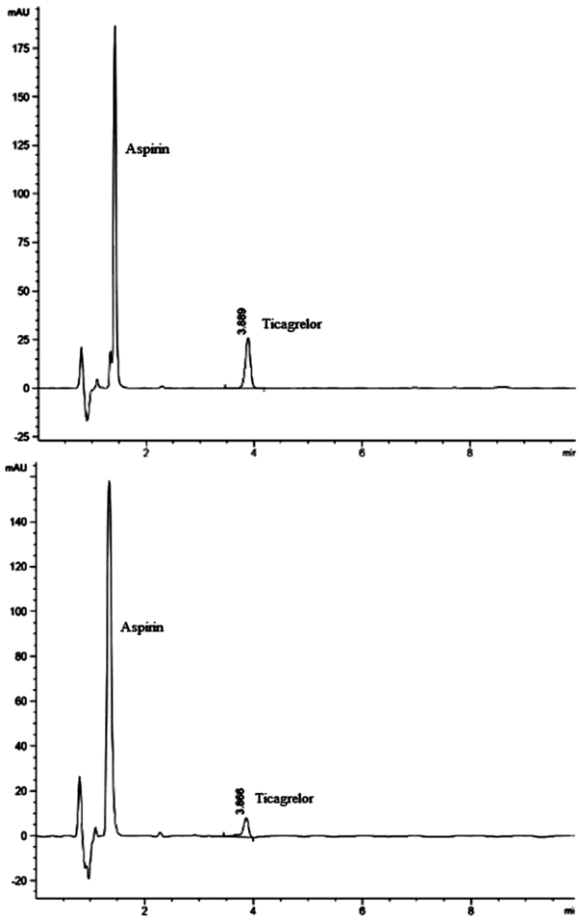

Fig. 2 — a) Chromatogram of co-crystal in SGF after 60 min and b) Chromatogram of co-crystal in blank FASSIF after 60 minutes

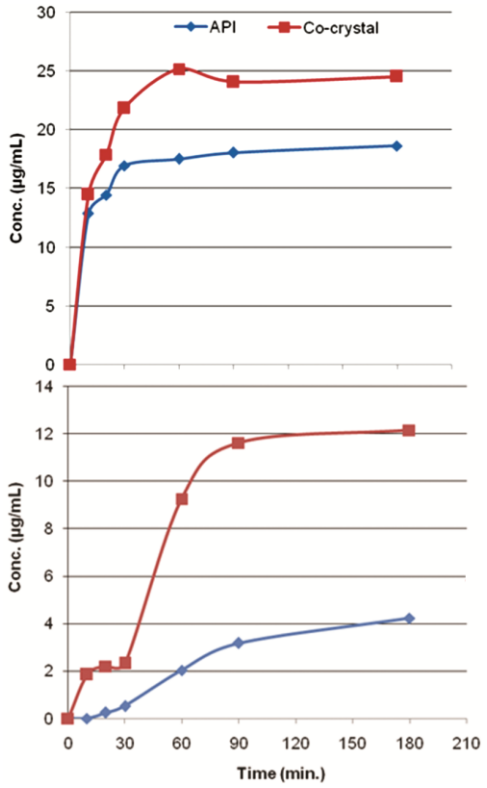

Fig. 3 — a) Comparative dissolution data of Ticagrelor hydrochloride and its co-crystal with sodium salt of Aspirin in SGF and b) Comparative dissolution data of Ticagrelor hydrochloride and its co-crystal with sodium salt of Aspirin in FASSIF

Table 2 — Dissolution data in SGF

Time (Minutes)	00	10	20	30	60	90	180
Conc. of API (ppm)	00	12.86	14.45	16.94	17.51	18.06	18.62
Conc. of Co-crystal (ppm)	0.0	14.48	17.89	21.84	25.17	24.07	24.50

Table 3 — Dissolution data in FASSIF

Time (min)	00	10	20	30	60	90	180
Conc. of API (ppm)	0.0	0.0	0.24	0.52	2.03	3.18	4.22
Conc. of Co-crystal (ppm)	0.0	1.84	2.20	2.31	9.25	11.61	12.14

Conclusion

Based on experimental data we conclude that a pharmaceutical co-crystal of platelet aggregation inhibitor; Ticagrelor hydrochloride with sodium salt of Aspirin has improved solubility characteristics in biorelevant media compare to API in free form. The finding proves better bioavailability of this BCS class IV drug as absorption of a drug from a solid dosage depends on the release of the drug substance from formulation, the dissolution of the drug under physiological conditions, and the permeability across the gastrointestinal tract. We believe that results obtained from this study would surely help in development of new formulations and also provides a base to predict the in vivo-in vitro correlation.

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