ENHANCEMENT OF SOLUBILITY OF A POORLY SOLUBLE ANTIPLATELET AGGREGATION DRUG BY COGRINDING TECHNIQUE

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ABSTRACT

Objective: The goal of this study was to formulate ticagrelor tablets with enhanced dissolution rate using cogrinding technique. However, it belongs to the biopharmaceutical classification system Class IV molecule, poorly soluble, and permeable.

Methods: Various ratios (0.2:1–1.67:1) of povidone (PVP)/drug were used in the formulations. Ticagrelor was cogrind with different percentage of PVP K 25 (carrier) in a mortar with a pestle for 30 min, then mixed with the rest excipients. A high-performance liquid chromatography stability indicating assay was developed to test the stability of ticagrelor in the selected formulae.

Results: The results showed that the presence of PVP in relatively high ratios compared to the drug is desirable for enhancing the dissolution rate of ticagrelor. The best-optimized formulae found were that F8 and F9 which showed good disintegration and dissolution rate of ticagrelor more than 92% after 30 min while the dissolution rate for ticagrelor standard was only 22% after 30 min. Stability studies were performed on the selected formulae F8 and F9.

Conclusion: The optimized formulae were evaluated for thickness, weight variation, hardness, friability, dissolution, and accelerated stability study for a period of 6 months. Cogrinding using PVP K 25 proved to enhance the dissolution of ticagrelor which may be due to the formation of a soluble complex between ticagrelor and PVP. The selected formulae F8 and F9 showed good stability.

Keywords: Ticagrelor, Stability indicating assay, Poorly soluble drug, Cogrinding

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INTRODUCTION

Ticagrelor is used for the prevention of thrombotic events in patients with acute coronary syndromes. The absolute bioavailability of ticagrelor is in the range 30–42% [1,2]. The chemical structure of ticagrelor is shown in Fig 1.

Ticagrelor is a crystalline powder with an aqueous solubility of approximately 10 µg/mL at room temperature. Ticagrelor exhibits no pKa value within the physiological range. Ticagrelor does not exhibit pH-dependent solubility. Its melting point is about 140°C–142°C as measured by differential scanning calorimetry. Partition coefficient of ticagrelor exhibits a log P (octanol/water) >4.0. Optical rotation of 1% w/v ticagrelor in ethanol is approximately −52° [3].

Ticagrelor belongs to the biopharmaceutical classification system (BCS) Class IV molecule which means that it is poorly soluble and has low permeability [4]. Hence, it is important to increase its solubility. Hence, dissolution rate and bioavailability will increase with improvement of the dissolution rate of the drug.

Furthermore, nanocrystals, spray drying, chitosan-based solvent change approach, preparation of dry elixir, and amorphous systems are used to enhance the dissolution rate of poorly soluble drugs [7]. Co-grinding is one of the solid dispersion techniques but it has some additional advantages over other approaches as it is simple, cheap, does not require any toxic organic solvents [8] and can be applied easily in the industry. Cogrinding technique has already been applied to some drugs such as phenytoin [9], furosemide [10], and glibenclamide [11]. However, the method of cogrinding for the preparation of ticagrelor has not been reported in the literature. Povidone (PVP) was chosen as a carrier for its capability of forming water-soluble complex with some poorly soluble drugs [12].

MATERIALS AND METHODS

Materials
Ticagrelor purchased from Virdev, India, lactose direct compression (lactose DC), croscarmellose sodium (Ac-di-sol), magnesium stearate and PVP K 25; kindly supplied by Elkahira Pharmaceutical Chemicals Company, Shoubra, Cairo, Egypt. Acetonitrile for high-performance liquid chromatography (HPLC) was purchased from Merck, Darmstadt, Germany. Hydrochloric acid, phosphoric acid, potassium dihydrogen phosphate, sodium hydroxide pellets, and hydrogen peroxide 30% were purchased from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt. High purity water was prepared using a Waters Milli-Q plus purification system.

Methodology
A stability indicating assay is developed and validated to determine drug content of ticagrelor and in stability studies. Furthermore, UV-VIS method was developed to determine percent dissolved of ticagrelor in fresh samples.

Development and validation of a HPLC stability indicating assay
Preparation of standard solution
40 mg of ticagrelor was weighed accurately and transferred into a 100 ml measuring flask, 50 ml methanol was added, shaken to dissolve by sonication for 5 min, completed to the volume with methanol and mixed well.

Preparation of test solution
Ten tablets were weighed, and the average weight of one tablet was found out and ground. A weight of powdered tablet equivalent to the
weight of one tablet was transferred into a 100 ml volumetric flask, about 50 ml methanol was added, sonicated for 15 min, completed to volume using methanol, mixed well and filtered through 0.45 μm membrane filter.

**Chromatographic conditions**

The separation was achieved using a Kromasil C18 (5 μm, 25 cm × 4.6 mm) column, a Waters HPLC apparatus consisting of pump 1525 and a UV/VIS detector 2487. The injection volume was 20 μl and a mobile phase consisting of phosphate buffer pH 3:acetonitrile (50:50 v/v) while at a flow-rate of 1.0 ml/min. Ticagrelor was detected at 254 nm. Linearity range was 30–90 μg/ml. The method was validated.

**Method validation**

The developed method was validated to establish the specificity, precision, linearity, accuracy, and robustness according to the United States Pharmacopeia (USP) [13], Food and Drug Administration (FDA) [14], and International Conference Harmonization (ICH) guidelines [15].

**Linearity**

The validation curve of the drug concentrations was constructed. The regression equation, the correlation coefficient, slope of the regression line, and residual standard deviation (σ) were calculated.

**Specificity**

The terms specificity and selectivity are often used interchangeably as both the USP and ICH [16] currently use the term specificity, it will also be used here to avoid any confusion. The USP [13] defines specificity as the ability to measure accurately and specifically the analyte of interest in the presence of other components in the sample matrix. These components may include other active ingredients, excipients, impurities, and degradation products.

**Stress degradation studies**

All the reagents used for degradation study (stressors), i.e. 0.1 N HCl, 0.1 N NaOH, and 30% (w/v) H₂O₂. The drug was subjected to forced degradation until optimum degradation (10–30%) was achieved.

**Accuracy**

Accuracy is the measure of the closeness of the experimental value to the true value. It should be established across the specified range (that is, the line of working range) of the analytical procedure.

**Precision**

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions or “the degree of agreement among individual test results obtained by repeatedly applying the analytical method to multiple samplings of a homogeneous sample.”

**Repeatability**

Six test solutions having all 100% of the test were prepared following the test preparation procedure in the analytical method. The results obtained were calculated, relative standard deviation (RSD) should not be more than ±1 as FDA [14] recommendations.

**Ruggedness (Intermediate precision)**

Intermediate precision expresses within-laboratory variations. This was previously evaluated as part of ruggedness to evaluate the reliability of the method. The data obtained from the interday and intraday confirm the ruggedness of the used analytical method.

**Robustness**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in some parameters and provide an assurance of its reliability during normal usage. The robustness of the method is investigated by varying some or all conditions, for example, the organic composition of the mobile phase, pH, ionic strength, column temperature, the age of column, and column type. ICH [15] guidelines recommend that robustness studies should be performed during the method development stage. Robustness can also be partly assured by good system suitability specification. Therefore, it is important to set tight but realistic system suitability specifications. Robustness of the method was investigated by changing the instrumental conditions such as flow rate (±10%), column oven temperature (±5%), the wavelength of detection (±5 nm), organic content in mobile phase (±2%), and pH of the buffer in mobile phase (±0.2 units).

**The lower limit of detection (LOD)**

The lower LOD is the lowest concentration of the analyte that can be detected, but has not necessarily quantitated, under the stated experimental conditions and could be calculated according to the formula:

\[
LOD = 3.3\sigma + S
\]

Where σ is the standard deviation of residuals, S is the slope of the line of the calibration curve [16].

**The lower limit of quantitation (LOQ)**

The lower LOQ is the lowest concentration of the analyte that can be determined with acceptable precision and accuracy under the stated experimental conditions of the method could be calculated according to the formula:

\[
LOQ = 10\sigma + S
\]

Where σ is the standard deviation of residuals, S is the slope of the line of the calibration curve [16].

**System suitability**

The system suitability specifications are parameters that provide assistance in achieving this purpose. According to the ICH [15] and the USP [13], system suitability tests are performed before the analysis of the actual samples. Parameters required, such as tailing factor (T) should be for ticagrelor not be more than 2, resolution (RS) should be more than 2 if there is more than one peak, capacity factor (K) should be more than 2, and plate count should be not <2000 [17,18] Figs. 3 and 2.

**UV-VIS method development**

UV-VIS method developed for the determination of ticagrelor in dissolution rate of ticagrelor fresh tablets.

**Standard solution preparation**

200 mg of ticagrelor was accurately weighed and transferred into 100 ml volumetric flask, 5 ml methanol was added and shaken for 5 min
to dissolve, completed to the volume using 0.3 % tween 80 in 0.1 N HCl, mixed well then diluted to give serial concentrations of ticagrelor in the range of 2–12 μg/ml in 0.3% tween 80 in 0.1 N HCl, scanned in the range of 200–500 nm (Shimadzu UV 1700 system in the range of 200–500 nm with 1 cm matched quartz cells) using 0.3% tween 80 in 0.1 N HCl as a blank, and λmax 298 nm was chosen to be the maximum λmax.

Preparation of test solution
Ten tablets were weighed, and the average weight was found out of one tablet, ground. A weight of powdered tablet equivalent to the weight of one tablet was transferred into a 100 ml volumetric flask, about 5 ml methanol was added and shaken for 10 min, completed to volume using 0.3% tween 80 in 0.1 N HCl, mixed well and filtered through 0.45 µm membrane filter. Then, diluted to give serial concentrations of ticagrelor in the range of 2–12 μg/ml in 0.3% tween 80 0.1 N HCl, measured at λmax 298 nm which was predetermined. The percent of ticagrelor was determined by substituting the regression equation Figs, 4 and 5.

Tablets preparation
Ticagrelor was coground with different percentage of PVP K 25 (carrier) in a mortar with a pestle for 30 min and sieved on a sieve mesh size 125 μm then was mixed with lactose DC, sieved through a 600 μm sieve and mixed for 3 min. Then, the previous powder blend was mixed with Ac-Di-Sol sieved through a 600 μm sieve and mixed for 3 min. Finally, lubricant (magnesium stearate) was sieved through a 600 μm sieve and mixed with the previous blend for 1 min.

The tablets were compressed on flat punch 9 mm. All tablets were compressed into 300 mg using a single punch tablet machine (Erweka, Germany). The force of compression was kept constant throughout the compression process (Table 1).

Evaluation of post-compression properties
Weight variation
Twenty tablets, from each formula, were individually weighed (Sartorius, Gottingen, Germany). The mean weight of tablets was calculated [14].

Content uniformity
The uniformity of content was determined by crushing 10 tablets from each formula and determining the drug content of each tablet individually using the developed HPLC method [13].

Friability
Ten tablets from each formula were accurately weighed and placed in the drum of a friablator (Pharma Test, Germany), which rotated at 25 rpm for a period of 4 min. The tablets were then brushed and reweighed. The percentage loss in weights was calculated and taken as a measure of friability [13].

Hardness
Ten tablets from each formula were tested for their hardness (Table Hardness Tester, Erweka, Germany). The mean hardness in kilograms was then determined [13].

Disintegration time
The disintegration time for each of six tablets from each formula was determined using USP Disintegration Tester (USP Disintegration, Pharma Test, Germany) [13].

In vitro dissolution studies
The test was performed in 0.3% tween 80 in 0.1 N HCl solution at a temperature of 37°C ±0.5 using the USP Dissolution Tester (Dissolution Apparatus Validata SR 6, Hanson Research Corporation, USA). Apparatus II (paddle), at a rotation of 75 rpm [19]. Aliquots, each of 5 ml of the dissolution medium were withdrawn at 5, 10, 20, 30, and 45 min intervals. The samples withdrawn were then filtered, adequately
Table 1: Formulae of ticagrelor prepared tablets

<table>
<thead>
<tr>
<th>Formula</th>
<th>Ticagrelor (mg)</th>
<th>Povidone (mg)</th>
<th>Carrier/drug ratio</th>
<th>Lactose DC (mg)</th>
<th>Ac-di-sol (mg)</th>
<th>Mg stearate (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>90</td>
<td>18</td>
<td>0.2:1</td>
<td>156</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>F2</td>
<td>90</td>
<td>27</td>
<td>0.3:1</td>
<td>147</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>F3</td>
<td>90</td>
<td>36</td>
<td>0.4:1</td>
<td>138</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>F4</td>
<td>90</td>
<td>45</td>
<td>0.56:1</td>
<td>129</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>F5</td>
<td>90</td>
<td>54</td>
<td>0.6:1</td>
<td>120</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>F6</td>
<td>90</td>
<td>63</td>
<td>0.7:1</td>
<td>111</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>F7</td>
<td>90</td>
<td>72</td>
<td>0.8:1</td>
<td>102</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>F8</td>
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<td>90</td>
<td>1:1</td>
<td>84</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>F9</td>
<td>90</td>
<td>120</td>
<td>1.33:1</td>
<td>54</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>F10</td>
<td>90</td>
<td>140</td>
<td>1.56:1</td>
<td>34</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>F11</td>
<td>90</td>
<td>150</td>
<td>1.67:1</td>
<td>24</td>
<td>30</td>
<td>6</td>
</tr>
</tbody>
</table>

*The values represent mean±standard deviation, n=5

Fig. 6: Dissolution profile of ticagrelor from prepared tablets (values represent mean±standard deviation, n=6)

diluted and analyzed for ticagrelor content by measuring absorbance at 298 nm using 0.3% tween 80 in 0.1 N HCl as a blank. A similar volume of 0.3% tween 80 in 0.1 N HCl was added to the dissolution medium to maintain sink conditions, and correction factor was included [20].

Stability studies
Stress testing should be done at 40°C/75% RH for 6 months. If a significant change occurs at these stress condition, then the formulation should be tested at 30°C/65% RH for 6 months as intermediate conditions. Stability studies should be done on tablets in its final package.

RESULTS AND DISCUSSION
The method was found to be specific, precise, sensitive, and stability indicating. The correlation coefficient (r) was found to be >0.999 which was within the limits specified (NLT 0.99) showed good linearity. The recovery was found to be in the range of 98–102% indicates that this method can be used for quantitative routine quality control analysis of pharmaceutical dosage forms. The precision of a method determines the closeness of agreement between a series of measurements of the same sample. The RSD values were found to be 0.1%–0.12 %. These values were well within the generally accepted limit of <2%. Hence, confirming the good precision of the assay method. The sensitivity of the method was measured by calculating the LOD and limit of quantification [21]. LOD and LOQ were calculated using GraphPad InStat® to be 2.97 μg/ml and 9 μg/ml, respectively.

The UV-VIS method was applied and showed to be suitable for determination of ticagrelor dissolved percent in fresh samples. Formulations post-compression properties were shown in Table 2. All Formulations were evaluated for weight variation and results indicated very low weight variation which lies within pharmacopeia limits, i.e. ±5%. Hardness was seen to be in the range of values of 4.8–8.3 kg/cm², friability of all formulae was <1%, and the disintegration time of formulae F1–F9 tablets was in the range of 0.5–3 min.

The dissolution rate of ticagrelor from F7, F8, F9, F10, and F11 formulae where the ratios of PVP K 25 to ticagrelor ranged from 0.8:1 to 1.67:1, ticagrelor released was 81%, 92.1%, 93.5%, 92%, and 93.2%, respectively, after 30 min while the dissolution rate for ticagrelor standard which was only 22% was shown in Fig. 2.

The formule F8 and F9 were selected for being stability tested in its final package at 40°C/75% RH for 6 months and were shown to be stable all over the study using the developed validated proposed stability indicating assay.

In comparison with the previous studies of researchers such as Ramesh et al. [22] who enhanced the solubility and the dissolution rate of ticagrelor by solvent evaporation solid dispersion technique. The maximum improvement of the dissolution rate was 72.5% after 30 min while in this study the dissolution rate was more than 92% after 30 min in the formulae F8 and F9. Furthermore, Ramesh et al. [22] reached 95.2% drug content as the maximum result while in this work, the minimum drug content obtained was 98.57%.

Dabhi et al. [23] tried to increase the dissolution rate of ticagrelor using solid dispersion made by spray drying in which a solid dispersion.
containing 1:3 ratio of ticagrelor: Gelucire 50/13 (G50/3) showed a dissolution rate of only 84% of ticagrelor dissolved after 30 min. These comparisons proved that there is a significant improvement in our work, especially regarding the dissolution rate Fig 6.

CONCLUSIONS

This study showed that cogrinding technique in the ratios of PVP K 25 to ticagrelor 1:1 and 1.33 of F8 and F9 exhibited more than 92% of ticagrelor was dissolved after 30 min while the dissolution of pure ticagrelor was 22% only. These results may be due to the formation of a soluble complex between ticagrelor and PVP [12,24] However, in a point of PVP/drug ratio increased, the disintegration time increased suddenly which may be due to PVP binding effect [12] in the formulae F 10 and F 11.

AUTHOR’S CONTRIBUTIONS

There is one author only who performed all procedures, tests and write the manuscript.

CONFLICTS OF INTEREST

The author declares that there are no conflicts of interest.

REFERENCES