ABSTRACT

Objective: The main purpose of this study was to develop a simple, precise, rapid and accurate RP-HPLC method for the quantitative determination of ticagrelor in human plasma.

Methods: The separation was accomplished by the isocratic method by utilizing phenomenex C18 column on a Shimadzu binary gradient liquid chromatography system furnished with LC-20AD solvent delivery system, SPD-20A photo-diode array detector and 20 µl loop volume in a rheodyne injector. The analyte was extracted by protein precipitation in the involvement of diethyl ether as a protein precipitator. The mobile phase was developed for the estimation of the drug in human plasma consists of acetonitrile and methanol in the ratio of 60:40% v/v. Separation was done with a flow rate of 1 ml/min at a detection wavelength of 254 nm.

Results: Retention time was found to be 4.503 min with a run time 10 min. Linearity shows in a range of 20-100 µg/ml, with a correlation coefficient of 0.9992 respectively. Stability studies of ticagrelor in plasma were carried out by, short term stability, long term stability and bench top was developed for the estimation of the drug in human plasma consists of acetonitrile and methanol in the ratio of 60:40% v/v. Separation was done with a flow rate of 1 ml/min at a detection wavelength of 254 nm.

Conclusion: The outcomes were observed to be inside the knowledge of ICH guidelines. The prepared solution was injected in triplicate, and % RSD was measured. Acquired results demonstrate that proposed strategy can be effortlessly and advantageously applied for routine examination of ticagrelor in human plasma.

Keywords: Bioanalytical method, Reverse phase HPLC, Ticagrelor, ICH
**Linearity**

Linearity was studied by standard solutions at different concentration levels. The linearity range for ticagrelor was found to be 20-100 µg/ml. The regression equation was found to be $y = 35798x - 9772.3$ with a coefficient of correlation ($r^2$) 0.9992. The correlation coefficient for linearity is 0.9999. Since the result is approximately close to the true value, the method is indicated as highly significant.

![Fig. 1: Chromatogram of ticagrelor in human plasma 100 µg/ml](image1)

![Fig. 2: Calibration curve of ticagrelor in human plasma](image2)

**Table 1: Linearity of ticagrelor in human plasma**

| Concentration (µg/ml) | Area   |
|-----------------------|--------|
| 20                    | 750690 |
| 40                    | 1352882|
| 60                    | 2124666|
| 80                    | 2861693|
| 100                   | 3590697|

**Precision**

Precision was determined by carrying three replicates of concentration 40 µg/ml and performed intraday (within a day) and interday (day to day) studies. The percentage relative standard deviation (%RSD) was found to be less than 1%. For Intraday precision study, evaluation was carried out by injecting a standard solution at various time intervals and %RSD of Ticagrelor was found to be 0.56% and 0.51% shown in table 2 where inter-day precision was carried out in consecutive days with %RSD of 0.76% and 0.81% shown in (table 3). The %RSD can be reached up to 2%

Since the outcome is less than 1% it was found to be satisfactory, which indicates method is precise [9].

**Table 2: Intraday study of ticagrelor in human plasma**

| Days | Standard concentration | Peak area |
|------|------------------------|-----------|
| 1st day | 40 | Morning | Afternoon |
|       |            | 320815   | 3202864  |
|       |            | 3212878  | 3218728  |
|       |            | 3238005  | 3236005  |
| MEAN  |            | 3217899.33 | 3219199  |
| STD DEV |      | 18124.41 | 16575.51 |
| % RSD  |      | 0.56%    | 0.51%    |
Table 3: Interday study of ticagrelor in human plasma

| Standard concentration | Peak area | Day 1 | Day 2 |
|------------------------|-----------|-------|-------|
| 40                     |           | 1730997 | 1730789 |
|                        |           | 1742154 | 1742415 |
|                        |           | 1757249 | 1758924 |
| MEAN                   |           | 1743466.66 | 1744042.66 |
| STD DEV                |           | 13175.13 | 14137.94 |
| % RSD                  |           | 0.76% | 0.81% |

Limit of detection (LOD)

LD was determined by standard deviation method and slope of the calibration plot by using the formula 3.3*σ/S. It was observed to be 0.382µg/ml since the observed concentration is low, the method is sufficiently sensitive.

Limit of quantification (LOQ)

LOD was determined by standard deviation method and slope of the calibration plot by using the formula 10*σ/S. It was observed to be 1.158µg/ml. As the amount of analyte was found to be less, we can estimate the drug at very low concentration.

Stability of ticagrelor in human plasma

Short term stability

Short term stability was determined by storing concentration of 20 and 100 µg/ml over a period of 6 h at room temperature. Stability was determined by performing three replicates and calculated % RSD. It was found to be 0.12% and 0.08% respectively, shown in table 4 as it is less than 1% the method is said to be stable.

Long term stability

Long-term stability was determined by storing concentration of 20 and 100 µg/ml over a period of 10 d at room temperature. Stability was determined by calculating the % RSD. It was found to be 0.18% and 0.15% respectively, as it is less than 1% the method can said to be stable.

Bench top stability

Three replicates of the lowest and higher concentration were determined. The samples were assessed after keeping at room temperature (bench top) against freshly prepared concentrations and %RSD was calculated [10]. It was found to be 1.19% and 1.30% respectively. The %RSD can be reached up to 2%. Since the result is less than 2% values were found to be satisfactory, which indicates method is stable.

Table 4: Short-term stability of ticagrelor

| Concentration | Peak area | Mean | Standard deviation | %RSD |
|---------------|-----------|------|--------------------|------|
| 20            | 1929188   | 1927512 | 2385.189 | 0.12% |
|               | 1922566   |       |                    |      |
|               | 1924781   |       |                    |      |
| 100           | 5491030   | 5495356 | 4249.185 | 0.08% |
|               | 549513    |       |                    |      |
|               | 5499524   |       |                    |      |

Table 5: Long-term stability of ticagrelor

| Concentration | Peak area | Mean | Standard deviation | %RSD |
|---------------|-----------|------|--------------------|------|
| 20            | 1152882   | 1153686 | 2065.306 | 0.18% |
|               | 1156032   |       |                    |      |
|               | 1152143   |       |                    |      |
| 100           | 4891697   | 489020  | 7398.169 | 0.15% |
|               | 4882466   |       |                    |      |
|               | 4897096   |       |                    |      |

Table 6: Bench top stability of ticagrelor

| Concentration | Old     | Fresh   | % RSD |
|---------------|---------|---------|-------|
| 20            | 1077812 | 1096036 | 1.19% |
| 100           | 5342790 | 5442090 | 1.30% |

CONCLUSION

The developed RP-HPLC method was found to be simple, precise, accurate and sensitive for the estimation of Ticagrelor in human plasma. Validation of results according to ICH and EMA carried out revealed high accuracy and good precision. The RSD for every one of the parameters are observed to be short of what one, which shows the legitimacy of the technique is reasonably fine. A mixture of acetonitrile and methanol mobile phase ratio of 60% v/v in pump A and 40% v/v in pump B at a flow rate of 1 ml/min. The wavelength was found to be 254 nm in U. V spectroscopy. Retention time was found to be 4.503 min with a run time 10 min. 20-100 µg/ml concentration of ticagrelor shows linearity with a correlation coefficient of 0.9992. Thus we can conclude that this method can be easily and conveniently adopted for the quality control analysis of ticagrelor in human plasma.

CONFLICTS OF INTERESTS

Declare none

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How to cite this article

- Delma D’cruz, Anu Babu, Eena Joshy, Aneesh TP. Bioanalytical method development and validation of ticagrelor by RP-HPLC. Int J Appl Pharm 2017;9(3):51-54.