RESEARCH ARTICLE   Impact Factor: 7.014

SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF TICAGRELOR IN BULK DRUG AND THEIR DOSAGE FORMS BY USING HYDROTROPIC AGENT

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ABSTRACT

A novel, simple, fast and reproducible UV spectrophotometric method was developed using 2M sodium acetate as hydrotropic solubilizing agent for the estimation of poorly water soluble drug Ticagrelor in bulk and in pharmaceutical dosage form. There was more than 58 fold enhancement in the solubility of ticagrelor in 2M sodium acetate solution as compared to solubility in distilled water. Various organic solvents such as methanol, chloroform, dimethyl formamide and acetonitrile have been employed for solubilization of poorly water-soluble drugs to carry out spectrophotometric analysis. Drawbacks of organic solvents include their higher cost, toxicity and pollution. Hydrotropic solubilization may be a proper choice to preclude the use of organic solvents. It involves the addition of large amount of a second solute to increase the aqueous solubility of the first solute. Ticagrelor exhibits absorption maximum at 256 nm. Sodium acetate did not show any absorbance above 240 nm and thus no interference in the estimation of drug was seen. Beer's law was found to be obeyed in the concentration range of 5-25μg/ml. In this method, there is no interference from any common pharmaceutical additives and diluents. The correlation co-efficient (' r2 value) for ticagrelor was 0.999.The results of analysis have been validated as per ICH guidelines. The percentage recovery of ticagrelor ranged from 97.84 to 100.16% in pharmaceutical dosage form. Results of the analysis for accuracy, precision, LOD, LOQ and were found to be satisfactory. Therefore the methods can be used for routine monitoring of ticagrelor in industry in the assay of bulk drug and tablets.

Keywords: Ticagrelor, Hydrotropy, Sodium acetate, Spectrophotometry

INTRODUCTION:

In the formulation development fields and pharmaceutical analysis, increasing the aqueous solubility of insoluble and slightly soluble drugs is major importance. Most of the newly developed drug molecules are lipophilic in nature and poor solubility is one of the frequent problems encountered. It is well known that drug efficacy can be sternly limited by poor aqueous solubility. It is also known that the side effects of some drugs are the consequence of their poor solubility. The ability to increase aqueous solubility can thus be a valuable assist to increasing effectiveness and/or reducing side effects for certain drugs. Various organic solvents like methanol, chloroform, alcohol, acetone, dimethyl formamide and benzene have

been employed for the solubilisation of poorly water soluble drugs for spectrophotometric estimations. Drawbacks of organic solvents include higher cost, toxicity, pollution and error in analysis due to volatility [1-2]. There are various approaches for solubilisation of poorly water soluble drugs. Hydrotropy is a solubilisation technique in which addition of large amount of a second solute results in an increase in the aqueous solubility of another solute. The hydrotropic agents are defined as non-micelle-forming substances, either liquids or solids, organic or inorganic, capable of solubilising insoluble compounds. On the other hand, planarity of the hydrophobic part has been emphasized as an important factor in the mechanism of hydrotropic solubilisation. Literature review shows that a large number of poorly water-soluble drugs like frusemide, cefixime, tinidazole, amlodipine besylate, pramipexole, torsemide using hydrotropic solubilising agents [3-8]. Concentrated aqueous solutions of a large number of hydrotropic agents like sodium benzoate, sodium salicylate, urea, sodium ascorbate, niacin amide and sodium citrate have been employed to enhance aqueous solubility of various poorly water-soluble drugs. The UV-Visible spectroscopy is a common analytical technique for quantitative and qualitative analysis of a sample which worked on Lambert- Beer's law. Ticagrelor (Fig 1) is orally active, reversibly binding P_2Y_{12} antagonist inhibiting platelet aggregation via P_2Y_{12} ADP-receptor[9]. Chemically, it is (1S,2S,3R,5S)-3-[7-{[(1R,2S)-2-(3,4-difluorophenyl)cyclopropyl]amino}- 5-(propylthio)-3H-[1,2,3]-triazolo[4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxycyclo pentane1 ,2-diol) [10]. Ticagrelor lowers the risk of thrombotic cardiovascular events in patients suffering from acute coronary syndrome [11]. Ticagrelor and its major metabolite reversibly interact with the P_2Y_{12} ADP-receptor to slow down platelet aggregation and thrombus formation in atherosclerotic disease [12-16]. Literature survey revealed that few HPLC, LC-MS and UV spectrophotometric methods have been developed and reported for the estimation of ticagrelor [17-21], However no efforts have been reported for the UV spectrometric method of ticagrelor in bulk form by using hydrotropic agent as a solvent, which could be very economic and easily applicable as well. Hence an attempt has been made to develop and establish a novel, simple, rapid and sensitive UV spectrometric method in accordance with ICH guidelines for the estimation of ticagrelor in bulk drug and formulation by using 2M sodium acetate as hydrotropic agent to increase the solubility of drug.
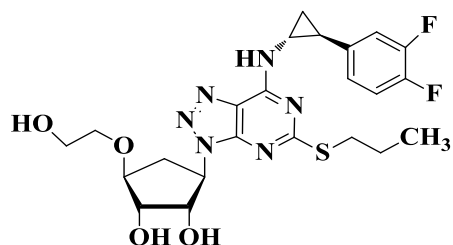

Fig. 1 Chemical structure of ticagrelor

EXPERIMENTAL

Apparatus

The proposed work was carried out on a Lab India UV-visible spectrophotometer (model 3000+ series), having double beam detector configuration. The absorption spectra of reference and test solution were carried out in a 1 cm quartz cell over the range of 200-800 nm.

Reagents and standards

The working standard of Ticagrelor was provided as gift sample from Spectrum Labs, Hyderabad, India. The market formulation Brilinta tablets (Ticagrelor 90 mg) were procured from local market. Sodium acetate, urea, sodium benzoate of AR grade were obtained from S.D. Fine Chemicals Ltd, Mumbai, India. All solvents and reagents were of analytical grade. All the solutions were protected for light and were analyzed on the day of preparations. Triple distilled water was generated in house.

Preliminary solubility studies

Solubility of ticagrelor was determined at 28±1 °C. An excess amount of drug was added to a screw capped 25 ml of volumetric flask containing different aqueous systems viz distilled water, buffer of pH 6.4, buffer of pH 8.2, and 2M sodium acetate solution. The volumetric flasks were shaken mechanically for 12 hrs at 28±1°C in a mechanical shaker. These solutions were allowed to equilibrate for next 24 hrs and then centrifuged for 5 min at 2000 rpm. The supernatant liquid was taken for appropriate dilution after filtered through Whatmann filter paper #41 and analyzed spectrophotometrically against corresponding solvent blank. After analysis, it was found that the enhancement in the solubility of ticagrelor was found to be more than and 58 folds in 2M sodium acetate solution as compared to solubility studies in other solvents. This enhancement of solubility is due to the hydrotropic solubilisation phenomenon.

Determination of wavelength of maximum absorption (λmax)

The stock solution (100 μg/ml) was diluted to 10 times to give a solution of 10μg/ml and 5mL of this solution was taken in a cuvette and scanned from 200 to 400 nm with Lab India UV-visible spectrophotometer (model 3000+ series). The distilled water was used as the blank. Ticagrelor was found to absorb maximum radiation at 256 nm and spectra of ticagrelor were shown in Fig 2.
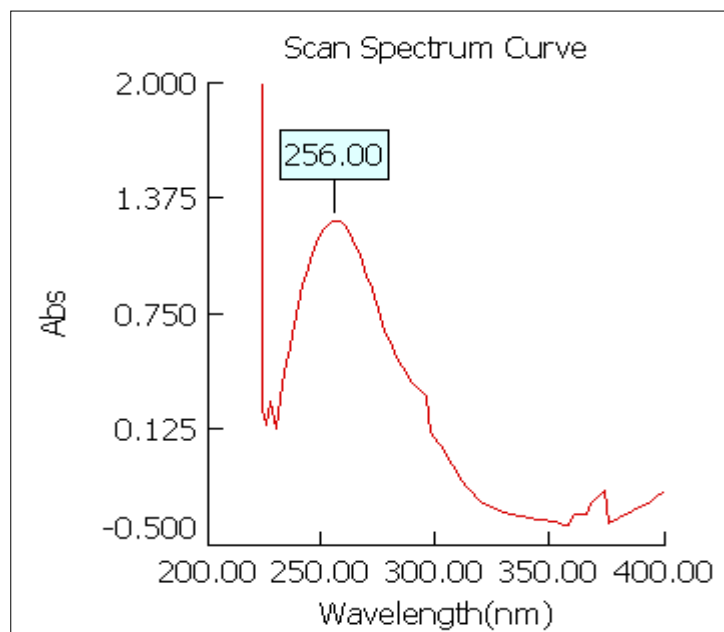
Preparation of calibration curve

Accurately weighed 100 mg of the ticagrelor drug sample were transferred in to 100 ml volumetric flask containing 10 ml of 2M sodium acetate solution and diluted up to 100 ml with distilled water. The standard solution (1000 µg/ml) was further diluted with distilled water to obtain 5, 10, 15, 20 and 25 µg /ml. Likewise the dilution ranging from 05-25 µg/ml were prepared in sodium acetate. Detection wavelength was selected for ticagrelor was 256 nm. Absorbance was noted against distilled water as blank. Calibration curve was plotted between concentration verses absorbance. Spectral data shown in Table-1 and calibration curve in Fig. 3.

Table 1 Optical characteristic and linearity data of ticagrelor

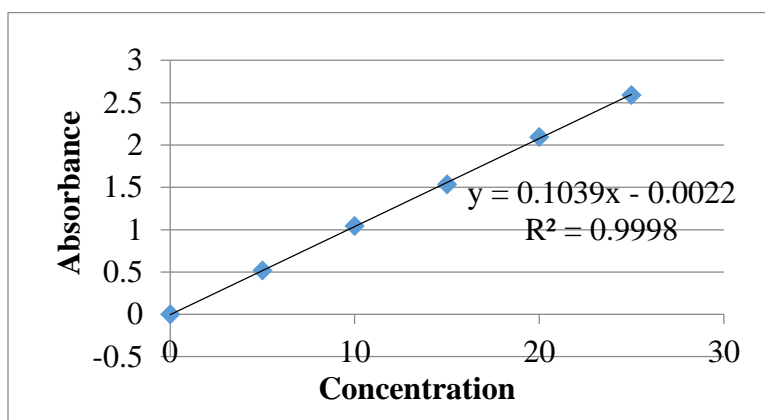| S. No.	Parameter	Ticagrelor
1	Working λ	256
2	Beer's law limit (µg/ml)	5-25
3	Correlation Coefficient (r2)*	0.999
4	Slope (m)*	0.103
5	Intercept (c)*	0.002
6	Number of samples (n)	15

*Average of five determination

Fig. 3 Calibration curve of ticagrelor at 256nm.

Analysis of tablet formulation

Marketed formulation Brilinta 90 mg (Astra Zeneca Pharmaceutical) was selected for tablet analysis. Twenty tablets of formulation were weighed and ground to a fine powder. An accurately weighed powder sample equivalent to 90 mg of ticagrelor was transferred to a 100 ml of volumetric flask containing 10 ml of 2M sodium acetate solution. The flask was shaken for about 10 min to solubilize the drug and then volume was made up to mark with distilled water. The solution was filtered through Whatmann filter paper No 41. The filtrate was diluted appropriately with distilled water and analyzed on UV spectrophotometer against reagent blank. Drug content of tablet formulation were calculated using calibration curve and results of statistical data shown in Table 2.

Table 2 Results and statistical parameters for tablet analysis

Drug	Label Claim (in mg)	Amount Found (in mg)	% Mean*	S.D.*	%COV*	Std. Error*
Ticagrelor	90	89.13	99.03	1.004	1.013	0.409
Ticagrelor	90	89.40	99.40	0.451	0.453	0.183
Ticagrelor	90	89.56	99.51	0.806	0.810	0.328

*Average of five determination

Validation

Linearity & Range

The linearity of calibration curves (Absorbance Vs concentration) in pure solution was checked over the concentration ranges of about 05-25 µg/ml of ticagrelor [22].

Accuracy

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. In preanalyzed tablet solution, a definite amount of drug was

added and then its recovery was studied. These studies were performed in by adding fixed amount of pure drug solution to the final dilution while varying the concentration of tablet sample solution in the final dilution. The percentage recovery and percentage relative standard deviation of the recovery were calculated and shown in Table 3.

Table 3 Result of recovery studies of tablet formulation with statistical evaluation

Drug	QC Conc (µg/ml)	Recovery Level % (Amount Drug Added)	Amount of Drug Found (Mean±SD)*	Coefficient of variation (%)	*Standard error
Ticagrelor	10	80	97.84 ±0.892	0.911	0.372
		100	98.73 ± 0.619	0.626	0.252
		120	99.54 ± 0.512	0.514	0.210
Ticagrelor	20	80	100.16 ± 0.843	0.841	0.344
		100	99.86 ± 0.793	0.193	0.323
		120	98.46 ± 0.734	0.745	0.297

*Average of five determination

Precision

To evaluate precision at different parameter like repeatability, intermediate precision and reproducibility, five dilutions in three replicates were analyzed in same day, in two different days and by two analysts for day to day and analyst to analyst variation. The %RSD values for Intraday and Interday precision were < 2%, indicating that the method was sufficiently precise. The results were shown in the Table 4.

Table 4 Results of precision

Parameters		Mean±SD*	%RSD
Precision	Repeatability	98.72±1.28	1.30
	Intermediate Precision		
	Day to Day	97.98±1.02	1.04
	Analyst to Analyst	98.49±0.12	0.12
	Reproducibility	99.91±0.72	0.72

*Average of five determination

RESULTS AND DISCUSSION

Based on the solubility, stability and spectral characteristics of the drug, 2M sodium acetate was selected as hydrotropic agent. After solubilising the ticagrelor in the selected hydrotropic agent, it was scanned in spectrum mode and 256nm was selected as wavelength for estimation, considering the reproducibility

and variability of the obtained result. The developed method was found to be linear in the range of 5 to 25μg/ml with correlation coefficient (r2) of 0.999 in sodium acetate and linear equation was Y=0.103X - 0.002. The mean percent label claims of tablets of ticagrelor in formulation estimated by the proposed method were found to be 99.03 to 99.51 in sodium acetate. These values are close to 100, indicating the accuracy of the proposed analytical method. Low values of standard deviation, percent coefficient of variation and standard error further validated the proposed method table 2. The values of mean percent recoveries were also found to be ranging from 97.84 ±0.892 to 100.16 ± 0.843 in sodium acetate. Also the values of standard deviation, percent coefficient of variation and standard error were satisfactorily low table 3. Result of precision at different level were found be within acceptable limits (RSD ‹ 2) table-4.

CONCLUSION

It was concluded that the proposed method is new, simple, cost effective, accurately, precise, safe and free from pollution and can be successfully employed in the routine analysis of ticagrelor in bulk drug and tablet dosage forms. Presence of hydrotropic agent do not shows any significant interference in the spectrophotometric assay thus further confirming the applicability and reproducibility of the developed method.

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