Different estimation method of Ticagrelor

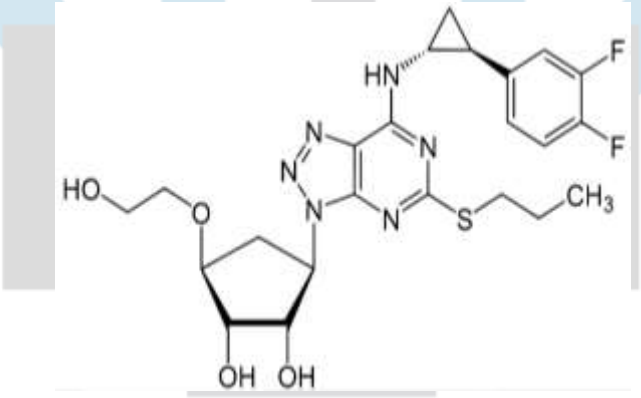
[1]Ashish kalyankar, [2]Dr. Uday Deokate

[1]Student, [2]Associate Professor
Department of pharmaceutical chemistry,
Government College of pharmacy,
Aurangabad, Maharashtra, India (431005)

Abstract: Ticagelor is a drug that prevents platelets from clumping together. It is used to prevent strokes, heart attacks, and other occurrences in persons who have acute coronary syndrome, which is a condition in which blood supply to the heart is disrupted. coronary arteries are the blood vessels in the heart. It works as a platelet aggregation inhibitor by inhibiting blood clotting. Blocking the P2Y 12 receptor It has a half-life of up to 12 hours. Hepatic metabolism and biliary excretion are two types of metabolism. Review gives the information about different method of estimation of Ticagelor. That includes the review on pharmaceutical analytical techniques like High-performance liquid chromatography, Reverse phase - High-performance liquid chromatography, Bio-analytical techniques, Spectrometry with the impurities, pharmaceutical dosage forms, bulk, and its formulations used for determination and validation. This review helps us to get updated information on validation of drug-Ticagelor till current date.

Keywords: Ticagelor, HPLC, U. V, validate, impurities

Introduction: Ticagelor is a drug that prevents platelets from clumping together. Ticagelor is a medication that is used to treat acute illness. Ticagelor blocks Adenosine Diphosphate (ADP) receptors direct, preventing signal transduction and platelet activation without hepatic activation 1-2. Ticagelor is a tiny molecule with a bodyweight of 522.568 and a chemical formula of C23H28F2N6O4S. Ticagelor chemical name is (1S, 2S, 3R, 5S) -3-[7-[[(1R, \s2S) -2-(3, 4-difluorophenyl) cyclopropyl]amino] -2-(3, 4-difluorophenyl) cyclopropyl]amino] -5- \spropyl sulfanyl triazolo[4,5-d] pyrimidin-3-yl] cyclopentane -5 (2-hydroxy-ethoxy) 2-diol-1, 2-diol-2-diol-2-dio 3. This conceptual found that drug may lower the risk of MACCE by lowering the incidence of stroke in Asian ACS patients. ticagelor did not show a significant effect on any and all, cardiovascular risk, MI, TVR, NACCE, or serious bleeding when opposed to antiplatelet. ticagelor has been linked to a significant increase in major/minor and mild bleeding problems**.** (1)
ticagelor has an about 8-hour plasma half-life, whereas the active metabolite has an approximately 12-hour plasma half-life. ticagelor chemical structure is shown below. (2)



TICAGRELOR

Pharmacokinetics: -it involves Absorption, Distribution, Metabolism, Excretion

Absorption: ticagelor is an orally administered, reversibly binding, and direct-acting P2Y12 receptor antagonist.

Distribution: ticagelor has volume of distribution 88L.

Metabolism: ticagelor is metabolized by the cytochrome P450 (CYP) enzyme to AR-C124910XX, a metabolite that possesses equivalent antiplatelet potency as the parent drug .

Excretion: The primary route of ticagelor elimination is hepatic metabolism 6. ticagelor is mainly excreted in the faeces, with renal excretion laying only a minor role; the primary route of excretion for the active metabolite is most likely biliary secretion

Pharmacodynamics: The inhibition of platelet aggregation (IPA) by ticagelor is acute and chronic platelet inhibition effects in response to 20 M ADP as the platelet aggregation agonist

Drug Interactions: ticagelor and AR-C124910XX are principally metabolized by CYP3A4 and, minor, by CYP3A5 enzymes. (3)

Mechanism of Action: ticagelor and its major metabolite reversibly interact with the platelet P2Y12 ADP-receptor to prevent signal transduction and platelet activation. ticagelor and its active metabolite are approximately equipotent

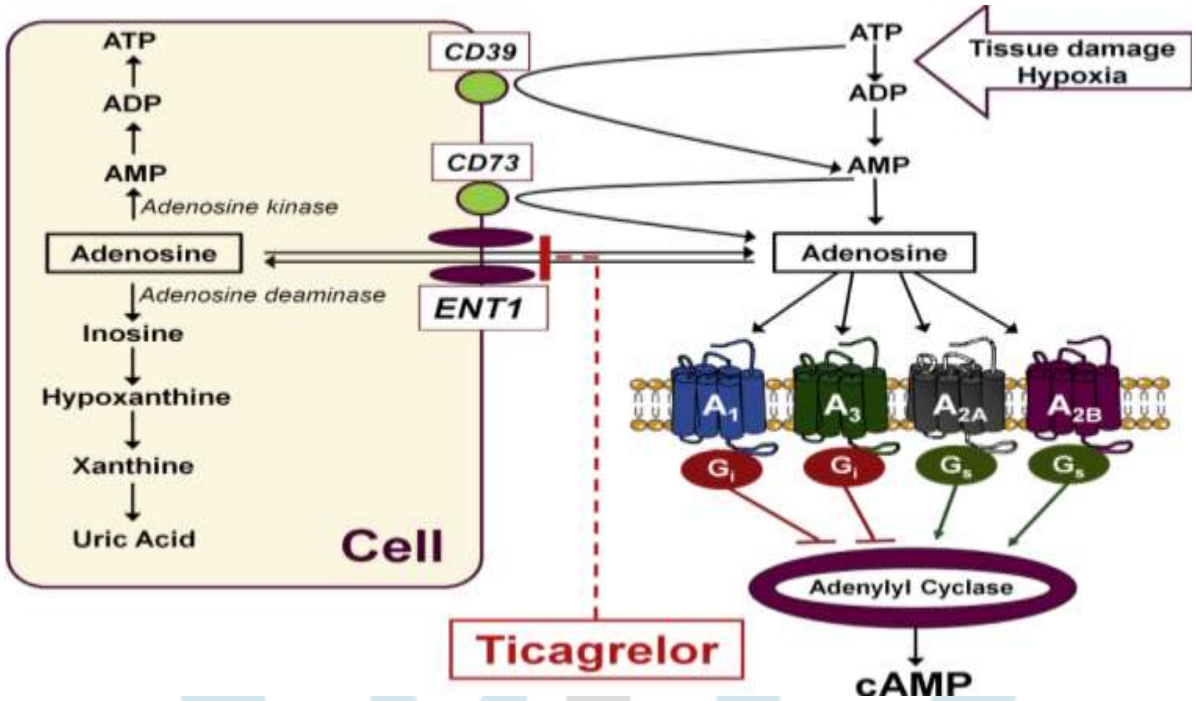


FIG. MECHANISM ACTION OF TICAGRELOR

➢ **Estimation method of ticagelor by UV spectrometry**: -

MATERIALS AND METHODS

Instrumentation

ticagelor absorbance was read using a double beam ELICO SL 210 UV spectrophotometer with two associated quartz cells and a one-meter light path. Weighing was done with an AJ balance (0.1 mg sensitivity). In this investigation, PCI Ltd., Mumbai, employed an Ultra sonication bath model no. 91250.

Chemicals and reagents

ticagelor was obtained from Hyderabad-based Hybrid Drugs Ltd. in Telangana, India. This research employed ticagelor (Brilinta) pills that had 90 mg of the indicated claim. E. Merck specialities, private Ltd., Mumbai, India provided the CH3OH..

Selection of the solvent

Many studies were carried out in order to determine the best solvent solution for disintegrating ticagelor. The stability of the medication was tested using solvents such as acetonitrile, DMSO, hexane, ethanol, methanol, and triple distilled water. ticagelor is accessible in a variety of solvents, including ACN, methanol, and DMSO. Because ticagelor exhibited the highest absorbance in methanol, it was used throughout the experiment.

Selection of detection wavelength

To establish the optimal ticagelor max, a 10 g/ml ticagelor solution in CH3OH was produced and scanned across the 200-400 nm wavelength range. The drug's highest absorbance was found to be at 255 nm when methanol and acetonitrile were employed as solvents. As a result, this wavelength was chosen as the subject of additional investigation**.**

Parameter	Results
Detection wavelength (λ_{max})	255 nm
Beer's law limits (µg/ml)	2-10
Molar absorptivity (L. mole^{-1} cm^{-1})	21407.82
Sandell's sensitivity (µg /cm^2/0.001 absorbance unit)	0.02441
Regression equation (Y = mx+ c)	0.0411x+0.0007
Slope (m)	0.0007
Intercept (c)	0.04111
The standard error of slope (S_m)	0.000159223
The standard error of intercept (S_c)	0.000964203
Standard error of estimate (S_e)	0.00133238
Correlation coefficient (r^2)	0.9999

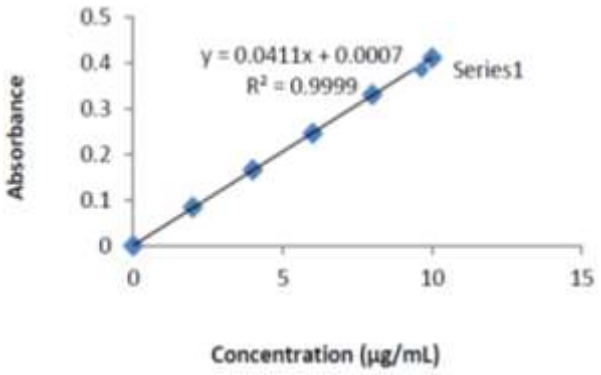
S.No	Concentration (µg/ml)	Absorbance
1	2	0.0838
2	4	0.1658
3	6	0.2458
4	8	0.3312
5	10	0.4112

Preparation of standard stock solution

Accurately weighed 100 mg of drug in 100 ml of diluent (methanol and acetonitrile-50:50) to get a 1000 µg/ml stock solution. Working standard solutions were further diluted to get a concentration range of 2-10 µg/ml. The final solution was prepared and further diluted to obtain 20µg/ml concentration.(4)

Preparation of Calibration curve

From the above prepared ticagelor stock solution, appropriate dilutions were prepared to get the final concentration of 2, 4, 6, 8, and 10 µg/ ml, and absorbance was taken at λ max 255 nm. Average of such five sets of values were taken for standard calibration plot, and the calibration curve was plotted. The aliquots of concentration ranging from 2-10 µg/ ml concentrations were used.



Method development and validation

Different types of solvents were tested for solubility Methanol, ethanol, DMSO, acetonitrile, and distilled water at 10 g/ml concentrations for ticagelor solvents. ticagelor, on the other hand, was soluble and stable in methanol and acetonitrile for at least 48 hours at ambient temperature. As a result, a diluent (50:50 methanol and acetonitrile) was employed to measure wavelength and prepare the standard and working concentrations. An assay of ticagelor 90 mg tablets was used at working concentration to check the planned technique of pharmaceutical manufacture was followed. The working concentration of the material was determined

using a 255 nm assay. The ICH Q2 (R1) guidelines17 for validation of analytical processes are used to validate a UV spectrophotometric technique. Linearity, specificity, accuracy, precision, robustness, LOD, and LOQ were all used to validate the procedure**.** (5)

For the measurement of TCG in bulk and dose forms, a simple UV spectrophotometric technique was established; samples were dissolved in a methanol: water (1:1, v/v) combination, and the determination wavelength was 222 nm; the analytical approach was verified according to ICH requirements. (6)

Linearity:

With 500 ppm of a solution (2–10 g/ml for UV technique), the curve was formed. Linear regression analysis, using the least square regression approach, was used to determine the linearity.

Accuracy:

ticagelor recovery studies at three different doses (50 percent, 100 percent) were used to assess the method's accuracy. and a hundred percent). In a triple investigation, UV concentrations of 6, 8, and 10 g/ml were tested. A certain quantity of previously studied data for the assessment of Ticagelor, a sample and a reference medication were added, and the recovery was evaluated. The proportion The mean percent recovery and recovery rate were computed.

Precision:

In terms of intraday and whole day precision, ticagelor was determined. Standard solution (6 g/ml for UV) was made from stock solution and the absorbance of the mixture were calculated six times (n=6) at two different times during the day for intraday precision analysis. By infusing the same volume of standard solutions into the system six times on successive days, the intraday precision was evaluated. Precision was calculated using the standard deviation and relative standard deviation. The RSD for peak locations should be NMT 2%, indicating that the developed is precise. (7)

	A	B	C	D	E	F	G	H	I
1	SUMMARY OUTPUT								
2									
3	*Regression Statistics*								
4	Multiple R	0.999970006							
5	R Square	0.999940014							
6	Adjusted R Square	0.999925017							
7	Standard Error	0.001332238							
8	Observations	6							
9									
10	ANOVA								
11		*df*	*SS*	*MS*	*F*	*Significance F*			
12	Regression	1	0.118343361	0.118343	66677.68223	1.34942E-09			
13	Residual	4	7.09943E-06	1.77E-06					
14	Total	5	0.11835046						
15									
16		*Coefficients*	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
17	Intercept	0.000714286	0.000964203	0.740804	0.499941621	-0.00196277	0.003391342	-0.00196277	0.0033913
18	X Variable 1	0.041117143	0.000159233	258.2202	1.34942E-09	0.040675042	0.041559244	0.040675042	0.0415592

Summery output of ticagelor

➢ **Estimation method of Ticagelor by RP-HPLC:-**

INSTRUMENTION: -

The Philips Infinity 1220, Infinity Fast-LC (Pressure limit up to 600 bars) with autonomous sampler and PDA detector was used to design and validate the chromatographic technique. The software Open Lab Chemo was used to test data gathering and processing**.** (8)

Method development and optimization of chromatographic conditions: -

A variety of mobile phases incorporating HPLC grade water, acetonitrile, and methanol in various ratios with or without buffers, as well as varied flow rates, were used for HPLC development. When the mobile phase included a combination of acetonitrile and methanol, an excellent uniform peak was detected. (50:50 % v\v) (5)

Preparation of standard stock solution (10 ppm) :- 1 mg of ticagelor reference standard was weighed accurately and transferred into a 100 ml volumetric flask. 30 ml diluent (acetonitrile: water 90:10 v/v) was added and the mixture was sonicated. The solution was diluted up to the mark with the diluent to give the standard stock solution. The λ max was determined using UV-VIS spectrophotometer. A working standard range from 2 to 7 ppm was prepared from the stock solution of 10 ppm and used for linearity studies. (9)

Preparation of mobile phase: -

Methanol and HPLC water in the ratio of 20:80 v/v was filtered through 0.45 µ membrane filter and degassed. Methanol was used as diluent. The mobile phase was filtered and sonicated before use. The flow rate of the mobile phase was maintained at 1.0 ml/min.

The detection of the drug was carried out at 254nm.

Recovery level (%)	Conc. taken(µg/ml)	Amount spiked(µg/ml)	Total amount	Amount found(µg/ml)	% recovery	Limit (98-102%)
50	5	2.5	7.5	7.56	102.17	Passed
100	5	5	10	9.91	99.10	Passed
150	5	7.5	12.5	12.58	100.64	Passed

Preparation of standard solution: -
From the pure sample, 100mg of ticagelor were accurately weighed and transferred into 100ml volumetric flask separately. They were dissolved in 100ml Methanol to obtain 1000µg/ml of stock solutions. From these stock solutions 1ml of ticagelor was taken into 10ml volumetric flasks separately and further diluted remaining with a methanol to get 100µg/ml concentration of ticagelor. The solutions were then filtered through 0.45µm Nylon filter**.**(10)

Linearity :-
Several aliquots of ticagelor standard solution were placed in separate 10 mL volumetric flasks and diluted up to the mark using diluents, resulting in final ticagelor concentrations ranging from 5 to 25 g/mL. The medicine was evaluated using a UV detector set to 252 nm, and the peak area for each peak was recorded. The correlation coefficient value of ticagelor was 0.9976. Within the concentration range specified, the data reveal a good connection between peak area and drug concentration.

Standard Solution	LOD µg/ml	LOQ µg/ml
Ticagrelor	0.2125	0.6440

System Suitability: -
System suitability parameters like retention time, theoretical plates and tailing factor were calculated and compared with standard values.

Accuracy :-
The recovery studies for the method were carried out by standard addition method. It was evaluated at three concentration levels (2.5, 5 and 7.5%) and the percentage recoveries were calculated.

Precision:- Intra- and inter-day precision experiments were used to determine the method's accuracy. This was tested by injecting three distinct ticagelor sample preparations from a single formulation at three different concentration levels on the same day (intra day) and three different days (Inter day). After that, the percent RSD was computed. The information is provided as a table**.**

Limit of Detection and Limit of Quantification: -
The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined based on the standard deviation of the response and the slope of the calibration curve. The sensitivity of the method was established by the LOD and the LOQ values.

Table 1: Data for Linearity

Concentration (µg/ml)	Area
5	567.98
10	1034.08
15	1583.81
20	2164.20
25	2771.20

Conc.µg/ml	Flow rate	RT	Mean area	SD	% RSD
10	0.9	4.1	1039.41	0.98	0.09
10	1.1	4.9	1016.86	14.36	1.41

Table 6: Change in Mobile Phase Composition

Conc.µg/ml	Mobile Phase	RT	Mean area	SD	% RSD
10	96:04	4.4	1003.23	15.78	1.5
10	94:6	4.6	1124.53	8.80	0.78

Table 7: Change in Wavelength

Conc.µg/ml	Wavelength (nm)	RT	Mean area	SD	% RSD
10	252	4.5	972.06	3.76	0.38
10	254	4.7	1683.67	8.80	0.52

Robustness: -

Robustness was established by introducing small changes in the HPLC optimized conditions which include mobile phase ratio (±1), flow rate ratio (±0.1) and wavelength (±1). This was studied using two replicates at a concentration level of 10µg/mL of ticagelor. **(11060)** We investigated the efficacy of ticagelor alone against ticagelor + aspirin on clinically significant bleeding in patients who were at high risk for bleeding or an ischemic episode and had had PCI in a double-blind experiment**.(8)**(11) In humans, ticagrelor is rapidly absorbed in the intestine and undergoes extensive metabolism to generate approximately ten different metabolites(12)

PHARMACOKINETICS: - the plasma concentration-time patterns of ticagelor and its main metabolite, ARC124910XX. ticagelor plasma level peaked 1 hour after a crushed tablet of 90 mg was delivered (median (range) t max was 1 (1–4) hour and 1 (1–3) hour when taken orally and through tube inserted, respectively). Peak plasma concentrations of 90 mg ticagelor were obtained 2 hours after oral administration as a complete tablet (median t max 2 (1–4) hours). With all treatments, circulating levels of ARC124910XX reached at around 2 hours (median t max 2 (1–8) hours) Broken ticagelor tablets delivered orally (148.6 ng/mL) or by nasogastric tube (264.6 ng/mL) had greater plasma concentrations of ticagelor at 0.5 hours post dose than whole-tablet delivery**.** (13)

Mechanism: - ticagelor (AZD6140) is the first reversible oral P2Y12 receptor antagonist that prevents platelet aggregation caused by ADP. Adenosine was the catalyst for the discovery of ticagelor (14)**.** To account for baseline and time-dependent confounders, weighted Cox proportional hazard models and robust variance estimates were used. (15) Between research in a systematic review, clinical and methodological variances are unavoidable. Indirect comparisons should be evaluated to see if the differences are big enough to produce intransitivity**.** (16) Clopidogrel is still considered as one of the most appropriate anti-platelet agent which is used along with aspirin post PCI. However, prasugrel and ticagrelor are newer emerging antiplatelet drugs which might be more potent in comparison to clopidogrel(17)

TICAGRELOR FOR THE TREATMENT OF ACUTE CORONARY SYNDROMES: -

P2Y12 blockers are potent antihypertensive drugs that work by inhibiting neutrophil P2Y12 sites. Aspirin and P2Y12 antagonists are advised as a treatment. In European recommendations, dual antiplatelet treatment regimens are recommended**.**(18) to minimise post interventional adverse events in patients with acute coronary syndrome (ACS) after pci . Drugs and prasugrel are irreversible thienopyridine prodrugs that block P2Y12 receptors, but ticagrelor is a temporary regulator that doesn't need bio activation. Despite the fact that both treatment and ticagelor are linked to a greater risk of severe bleeding, both ticagelor and prasugrel outperformed clopidogrel in preventing major adverse cardiac events in ACS patients [3, 4]. For Thromboembolic patients who need PCI, prasugrel is preferable to ticagelor.(15) Ticagrelor, an oral antiplatelet therapy has its function to prevent an atherothrombotic events in acute coronary syndromes(19)

conclusion :- in the tablet A simple, precise, accurate UV spectroscopic method was developed and validated for the estimation of Ticagrelor. This method was found to be economical in terms of usage of solvents and yet to sensitive compared to the existing methods. In this study, the precision and accuracy % RSD was < 2 % in all cases. This method provides reproducible results with high precision, accuracy, and was capable of analyzing Ticagrelor in low concentrations. However, this UV method is simple, quick, sensitive. Hence the developed method can be used in the regular quality control of Ticagrelor dosage form.

References

1. Galimzhanov AM, Azizov BS. Ticagrelor for Asian patients with acute coronary syndrome in real-world practice: A systematic review and meta-analysis of observational studies. Indian Heart J. 2019;71(1):15–24.
2. Azhakesan A. ANALYTICAL METHODS OF TICAGRELOR: A REVIEW G. Nikitha and A. Ajitha * Department of Pharmaceutical Analysis, Faculty of Pharmaceutical Analysis, CMR College of Pharmacy, Medchal, Hyderabad – 501401, Telangana, India. 2021;12(12):6260–9.
3. AstraZeneca UK. Ticagrelor for the Treatment of Acute Coronary Syndromes. NICE Single Technol Apprais (STA). 2010;(November).
4. Pandya D, Patel M, Ghediya R, Ranjan K, Shah A, Khunt R. UV-Vis spectrophotometric assay determination of oral antiplatelet ticagrelor drug in pharmaceutical formulation: Application to content uniformity. J Chem Pharm Res. 2016;2016(81):316–21.
5. Sankar R. 143.Ticagrelor UV. 2020;(October).
6. Kelemen H, Hancu G, Papp LA. Analytical methodologies for the determination of ticagrelor. Biomed Chromatogr. 2019;33(7):0–3.
7. Anil Kumar N, Rupa Naga Swathi P, Sharmila D, Sharmila S, Pawar AKM. A validated stability indicating method of UV-Spectrophotometry for the estimation of ticagrelor in bulk & marketed formulation. Der Pharm Lett. 2016;8(19):309–15.
8. Ambasana.M, Kapuriya.N.P M. K., Ladva.K.D. An Improved Assay Method for the Estimation of Ticagrelor Hydrochloride By Reverse Phase Liquid Chromatography. Int J Pharm Sci Res. 2016;7(5):2009–14.
9. Tabassum.K, Sarvesh.R. Analytical method development and validation studies of ticagrelor tablets by RP-HPLC. Int J Appl Pharm. 2017;9(4):10–21.
10. Shyamalambica.P, Lakshmi.M. Development and Validation of Stability Indicating Hplc Method for Estimation of Ticagrelor in Formulation. Int J Eng Res Technol. 2018;7(10):522–7.
11. Mehran R, Baber U, Sharma SK, Cohen DJ, Angiolillo DJ, Briguori C, et al. Ticagrelor with or without Aspirin in High-Risk Patients after PCI. N Engl J Med. 2019;381(21):2032–42.
12. Chae SU, Min KL, Lee C Bin, Huang Z, Chang MJ, Bae SK. Steady-State Pharmacokinetics in Patients. 2019;27(3):98–106.
13. Teng R, Carlson G, Hsia J. An open-label, randomized bioavailability study with alternative methods of administration of crushed ticagrelor tablets in healthy volunteers. Int J Clin Pharmacol Ther. 2015;53(2):182–9.
14. Tan Q, Jiang X, Huang S, Zhang T, Chen L, Xie S, et al. The clinical efficacy and safety evaluation of ticagrelor for acute coronary syndrome in general ACS patients and diabetic patients: A systematic review and meta-Analysis. PLoS One. 2017;12(5):1–18.
15. Belviso N, Aronow HD, Wyss R, Barbour M, Zhang Y, Wen X, et al. Comparative effectiveness and safety of prasugrel versus ticagrelor following percutaneous coronary intervention: An observational study. Pharmacotherapy. 2021;41(6):515–25.
16. Chaimani A, Caldwell DM, Li T, Higgins JP, Salanti G. Chapter 11: Undertaking network meta-analyses 11.1 What is network meta-analysis? 2021;(September):1–46.
17. Bundhun PK, Shi JX, Huang F. Head to head comparison of Prasugrel versus Ticagrelor in patients with acute coronary syndrome: A systematic review and meta-analysis of randomized trials. BMC Pharmacol Toxicol. 2017;18(1):1–9.
18. Fong LCW, Lee NHC, Yan AT, Ng MY. Comparison of Prasugrel and Ticagrelor for Patients with Acute Coronary Syndrome: A Systematic Review and Meta-Analysis. Cardiol. 2022;147(1):1–13.
19. Indonesia UI. AN ECONOMIC EVALUATION OF TICAGRELOR AS COST- EFFECTIVE DRUG FOR ACUTE CORONARY SYNDROMES: Australia Healthcare System Perspective Wulandari Berliani Putri. (Mi).