ejpmr, 2022, 9(6), 271-276

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DEVELOPMENT AND VALIDATION OF A NEW STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ASPIRIN AND TICLOPIDINE

Y. Himaja, G. Josephine, M. Aparna, Sowjanya G., *P. Sridevi, Vinutha K., M. Bhagavan Raju

Sri Venkateswara College of Pharmacy, Madhapur, Hyderabad.

***Corresponding Author: P. Sridevi**

Sri Venkateswara College of Pharmacy, Madhapur, Hyderabad.

Article Received on 26/03/2022 **Article Revised on 16/04/2022** **Article Accepted on 06/05/2022**

ABSTRACT

A new stability indicating RP HPLC method has been developed and validated for simultaneous estimation of Aspirin and Ticlopidine in bulk and dosage forms. The method involves separation on Xterra C18 column (250mm x 4.6mm x5µm particle size). The optimized mobile phase consists of 0.1% OPA (pH 2.8) and Methanol (45:55v/v) with a flow rate of 1ml/min and UV detection at 229mn. Retention time was 2.78 min (Aspirin), 3.71 min (Ticlopidine). Linearity range was 20-100 ug/ml (Aspirin), 60-300 ug/ml (Ticlopidine). Accuracy was in the range of 96.92-100.35% for both drugs. Precision was 0.8% and 0.5% for Aspirin and Ticlopidine. LOD and LOQ are 0.42ug/ml and 1.38 ug/ml for Aspirin, 0.27ug/ml and 0.89ug/ml for Ticlopidine. The method developed is more sensitive, accurate and precise than the methods reported earlier. Retention time and run time were also less and hence the method is economical. When applied for tablet assay, drug content was within 98.55-101.4 % of labelled content. Forced degradation studies indicated the suitability of the method for stability studies.

KEYWORDS: Aspirin and Ticlopidine, RP-HPLC Method, Simultaneous estimation, Validation, Forced degradation studies.

INTRODUCTION

Aspirin is also known as acetylsalicylic acid is a salicy-lates drug, often used as an analgesic, antipyretic, and anti-inflammatory and also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecule together to create a patch over damage of the walls within blood vessels. Chemically it is 2-acetoxybenzoic acid and is a nonsteroidal anti-inflammatory drug (NSAIDs) and shows inhibition of the enzyme cyclooxygenase and it is official in Indian Pharmacopoeia, The United States Pharmacopeia and British Pharmacopoeia.[1-4]

Ticlopidine Hydrochloride is an antiplatelet drug of the thienopyridine class. Chemically it is 5-(2- chlorobenzyl) 4,5,6,7-tetrahydrothieno [3,2-c] pyridine and it is official in British pharmacopoeia. It is an adenosine diphos-phate (ADP) receptor inhibitor, inhibits platelet aggrega-tion by altering the function of platelet membranes thus prolongs bleeding time.[1-5]

Literature survey revealed that there are various meth-ods have been reported for estimation of ASP such as UV, HPLC, HPTLC, GC, Fluorimetry individually and in combined dosage form with other drugs. For TIC various analytical methods have been reported for its individual estimation includes UV, HPLC, UPLC. Literature survey also reveals that no HPLC method is reported for simul- taneous estimation of TIC and ASP in combination therefore, in the present work, a successful attempt has been made to estimate both these drugs simultaneously by RP-HPLC, therefore the aim of the study was to de- velop simple, rapid, accurate, reproducible and eco- nomic Simultaneous estimation of TIC and ASP from its formulation.[6-16] The proposed methods were vali- dated as per the International Conference on Harmoni- zation (ICH) analytical method validation guidelines.[17] Aspirin is also known as acetylsalicylic acid is a salicy- lates drug, often used as an analgesic, antipyretic, and anti-inflammatory and also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecule together to create a patch over damage of the walls within blood vessels. Chemically it is 2-acetoxybenzoic acid and is a nonsteroidal anti-inflammatory drug (NSAIDs) and shows inhibition of the enzyme cyclooxygenase and it is official in Indian Pharmacopoeia, The United States Pharmacopeia and British Pharmacopoeia.[1-4]

Ticlopidine Hydrochloride is an antiplatelet drug of the thienopyridine class. Chemically it is 5-(2- chlorobenzyl) 4,5,6,7-tetrahydrothieno [3,2-c] pyridine and it is official in British pharmacopoeia. It is an adenosine diphos-phate (ADP) receptor inhibitor, inhibits platelet aggrega-

tion by altering the function of platelet membranes thus prolongs bleeding time.[1-5]

Literature survey revealed that there are various methods have been reported for estimation of ASP such as UV, HPLC, HPTLC, GC, Fluorimetry individually and in combined dosage form with other drugs. For TIC various analytical methods have been reported for its individual estimation includes UV, HPLC, UPLC. Literature survey also reveals that no HPLC method is reported for simul- taneous estimation of TIC and ASP in combination therefore, in the present work, a successful attempt has been made to estimate both these drugs simultaneously by RP-HPLC, therefore the aim of the study was to de- velop simple, rapid, accurate, reproducible and eco- nomic Simultaneous estimation of TIC and ASP from its formulation.[6-16] The proposed methods were vali- dated as per the International Conference on Harmoni- zation (ICH) analytical method validation guidelines.[17]

Aspirin is also known as acetylsalicylic acid is a salicylates drug, often used as an analgesic, antipyretic, and anti-inflammatory and also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecule together to create a patch over damage of the walls within blood vessels. Chemically it is 2-acetoxybenzoic acid and is a nonsteroidal anti-inflammatory drug (NSAIDs) and shows inhibition of the enzyme cyclooxygenase and it is official in Indian Pharmacopoeia, The United States Pharmacopeia and British Pharmacopoeia.[1-4] Ticlopidine Hydrochloride is an antiplatelet drug of the thienopyridine class. Chemically it is 5-(2- chlorobenzyl) 4,5,6,7-tetrahydrothieno [3,2-c] pyridine and it is official in British pharmacopoeia. It is an adenosine diphosphate (ADP) receptor inhibitor, inhibits platelet aggregation by altering the function of platelet membranes thus prolongs bleeding time.[1-5] Literature survey revealed that there are various methods have been reported for estimation of ASP such as UV, HPLC, HPTLC, GC, Fluorimetry individually and in combined dosage form with other drugs. For TIC various analytical methods have been reported for its individual estimation includes UV, HPLC, UPLC. Literature survey also reveals that no HPLC method is reported for simultaneous estimation of TIC and ASP in combination therefore, in the present work, a successful attempt has been made to estimate both these drugs simultaneously by RP-HPLC, therefore the aim of the study was to develop simple, rapid, accurate, reproducible and economic Simultaneous estimation of TIC and ASP from its formulation.[6-16] The proposed methods were validated as per the International Conference on Harmonization (ICH) analytical method validation guidelines.[17]

MATERIALS AND METHODS

Materials and reagents

HPLC water (Lichrosolv^{R}Merck Lifesciences Pvt.Ltd., Mumbai, India) Ortho phosphoric acid (Thermo Fischer Scientific Pvt Ltd., Mumbai, India) were used in the study. The working standards of Aspirin and Ticlopidine were generous gift obtained from Pharma Train Pvt Ltd., Hyderabad, India. Duraplus tablet containing Aspirin 10mg and Ticlopidine 30mg was kindly supplied by Sun pharmaceutical Industries Ltd.

Instrumentation

Chromatography was performed on a WATERS 2695 HPLC column (waters corporation, Mildord, USA) with an autosampler and equipped with a 2996 series of PDA detector with a spectral bandpass of 1.2nm. Components were detected using UV and that processing was achieved by Empower 2 software. A hot air oven was used for thermal degradation of the samples and a UV crossinker, with series of 23400 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 & 300nm was selected for photolytic degradation. Ultrasonic bath (Toshcon by Toshniwal), digital Ph meter (Systronics model 802) were used in the study.

Chromatography conditions

The chromatographic conditions was performed on XTerra C_{18} column (250 X 4.6mm,5µm particle size) at an ambient column temperature. The samples were eluted using 0.1%Ortho phosphoric acid (pH adjusted to 2.8):Methanol(45:55v/v) as the mobile phase at a flow rate of 1 ml/min and samples were degassed by ultra sonication for 20 min and filtered through 0.45µm Nylon(N66)47mm membrane filter. The measurements were carried out with an injection volume of 10µL, flow rate was set to 1.0 mL/min, and UV detection was carried out at 229nm. All determinations were done at ambient column temperature (30°C). The chromatograms of the prepared standard stock solutions of Aspirin and Ticlopidine were recorded under optimized chromatographic conditions (**Fig. 1**).

Diluent

Buffer and Methanol in 50:50 v/v ratio.

Preparation of Standard Solutions

Stock standard solution

Standard stock solution of Aspirin and Ticlopidine were prepared by dissolving 20 mg of Aspirin and 60mg of Ticlopidine in 10ml of diluent (Buffer: Methanol, 50:50v/v) in a 10ml clean dry volumetric flask and the standard solutions was filtered through 0.45µm nylon membrane filter and degassed by sonicator to get the concentration of 2000µg/ml of Aspirin and 6000µg/ml of Ticlopidine. The above standard stock solution suitably diluted with diluents to obtain working concentration drugs.

Working Standard Solution

Working standard solution of Aspirin and Ticlopidine were prepared by taking 0.3ml of stock solutions of Aspirin and Ticlopidine in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 60µg/ml of Aspirin and 180µg/ml of Ticlopidine.

Preparation of Sample Solutions of Aspirin and Ticlopidine

Twenty tablets were accurately weighed and powdered and tablet powder equivalent to 20 mg of Aspirin and 60 mg of Ticlopidine was taken into 10 ml clean dry volumetric flask, diluent was added and sonicated to dissolve completely and volume was made up to volume with the diluent. The above sample solution suitably diluted to get a concentration of 60µg/ml of Aspirin and 180µg/ml of Ticlopidine.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Aspirin and Ticlopidine were soluble in polar solvent, so the developed method of estimation was carried out on reverse phase high performance liquid chromatography. To develop a rugged and suitable Preliminary trials were taken with different composition of buffer and organic phase of mobile phases with pH range of 2.5–5. After evaluating all these factors, a XTerra C_{18} column was found to be giving satisfactory results. The selection of methanol and buffer were based on chemical structure of both the drugs. Best results were obtained with 0.1% O-phosphoric acid pH adjusted to 2.8 with sodium hydroxide solution that improved the peak shapes of Aspirin and Ticlopidine. Mobile phase composition consisting of a mixture of buffer-pH 2.8 (0.1% Ortho phosphoric acid): Methanol (45:55v/v). Flow rates between 0.5 to1.2ml/min were tried. Flow rate of 1ml/min was observed to be enough to get both the drugs eluted within less than 10min. Under above described experimental conditions, all the peaks were well defined and free from tailing. The concern of small deliberate changes in the mobile phase composition, flow rates, and column temperature on results were evaluated as a part of testing for methods robustness.

Validation of Method Developed

The proposed method was validated according to the ICH guidelines[12] for the following parameters.

System suitability

The Retention time of Aspirin and Ticlopidine using optimum conditions was 2.78min and 3.72min respectively. For two of them, the peak symmetries were <1.5 and the theoretical plates numbers were >2000 and %RSD of areas of six standard injections of Aspirin and Ticlopidine was less than 2. These values are within the acceptable range of United States pharmacopoeia definition and the chromatographic conditions. The results obtained are shown in **Table 1.**

Table 1: System suitability results of Aspirin and Ticlopidine.

Parameter	Aspirin	Ticlopidine
Peak area	188339 (0.46%)*	1092032 (0.14%)*
Theoretical plates	2675.81±0.302	3626.04±0.258
Retention time	2.789±0.031	3.726±0.057
Tailing factor	1.10±0.07	1.27±0.05

*RSD (%)

Specificity

The specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. Optimized chromatogram of Aspirin and Ticlopidine is shown in **Fig. 1** clearly shows the ability of the method to assess the analyte in the presence of other excipients.

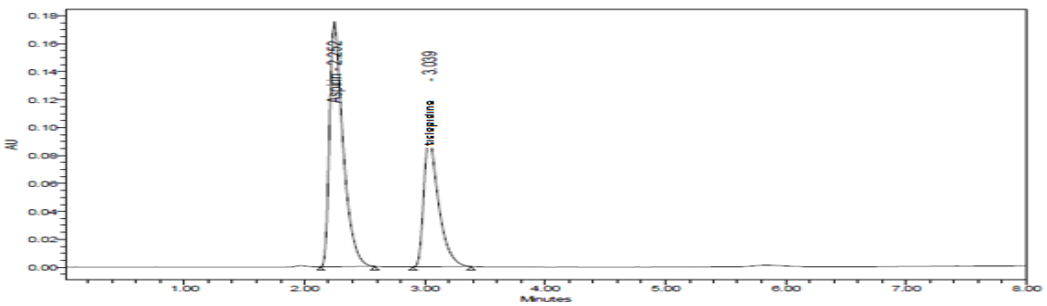

Fig. 1: Optimized Chromatogram of Aspirin and Ticlopidine.

Linearity and Range

Linearity was assessed for the two oral anti diabetic drugs at concentration ranges 20-100µg/ml for Aspirin and 60-300µg/ml for Ticlopidine. A linear relationship was established at these ranges between Area under the peak (AUP) and concentration. Good linearity was proved by high values of coefficient of determinations (**Fig.2 and Fig.3**). The results were tabulated in **Table 2**

Table 2: Linearity data of Aspirin and Ticlopidine.

Level	Concentration of Aspirin (µg/ml)	Peak area	Concentration of Ticlopidine (µg/ml)	Peak area
1	20	61652	60	352575
2	40	129447	120	713850
3	60	189375	180	1082534
4	80	244442	240	1408995
5	100	309206	300	1792376

Fig.2. Linearity graph of Aspirin.

Fig.3. Linearity graph of Ticlopidine.

Limit of Detection (LOD)/Limit of Quantitation (LOQ)

The LOD was determined on the basis of signal to noise ratios and was determined using analytical response of three times the background noise. LOQ was determined as the lowest amount of analyte that was reproducibly quantified above the baseline noise following triplicate injections. Both LOQ and LOD were calculated on the peak area using the following equations:

LOQ= 10 x N/ B

LOD= 3 x N/ B

The limit of detection and limit of quantification were evaluated by serial dilutions of Aspirin and Ticlopidine stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The LOD value for Aspirin and Ticlopidine was found to be 0.42 µg/mL and 0.27 µg/mL, respectively, and the LOQ value 1.386 µg/mL and 0.89 µg/mL, respectively.

Precision

System Precision

System Precision was carried to ensure analytical system is working properly. One dilution of both the drugs in six replicates was injected into HPLC system & was analyzed and the results were found within the acceptance limits (RSD<2).

Method Precision (Repeatability)

Precision is expressed as the closeness of agreement between a series of measurements obtaining from multiple sampling of the same homogeneous sample. Six replicate injections of a known concentration of sample preparation of Aspirin (60 µg/mL) and Ticlopidine (180 µg/mL) have been analyzed by injecting them into a HPLC column on the same day and on consecutive days. The results of precision are given in **Table 4**.

Table 4: Method Precision data for Aspirin and Ticlopidine.

Aspirin				Ticlopidine		
S. No	Concentration (µg/ml)	Peak Area	% Assay	Concentration (µg/ml)	Peak Area	% Assay
1	60	186186	98.8	180	1055440	100.22
2	60	184635	101.6	180	1060313	98.04
3	60	188760	98.4	180	1050116	100.29
4	60	188378	101.2	180	1061756	101.20
5	60	189416	100.9	180	1076488	101.24
6	60	191859	98.6	180	1095045	99.05
Average		188205.7	99.9	Average	1066526.3	100
SD		2528.9	1.5	SD	16529.5	1.3
%RSD		1.3	1.5	%RSD	1.5	1.3

Accuracy

The percentage recovery was calculated by preparing standard drug concentrations of Aspirin and Ticlopidine with concentration levels of 50%, 100% and 150%. Good recovery of the spiked drugs was obtained at each added concentration, and the mean percentage recovery of Aspirin and Ticlopidine was achieved between 97.44-100.35 ± 0.753% and 96.92-100.23±0.327. The results are given in **Tables 5,6**.

Table 5: Recovery data of Aspirin.

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S1:50%	30	29.6	98.66	Mean=97.44
S2:50%	30	29.2	97.33	S.D=1.16
S3:50%	30	28.9	96.33	%RSD=1.1
S4:100%	60	60.04	100.06	Mean=99.76
S5:100%	60	59.42	99.03	S.D=0.63
S6:100%	60	60.12	100.2	%RSD=0.6
S7:150%	90	90.54	100.6	Mean=100.35
S8:150%	90	90.28	100.311	S.D=0.22
S9:150%	90	90.14	100.15	%RSD=0.2

Table 6: Recovery data of Ticlopidine.

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S1:50%	90	90.43	100.47	Mean=96.92
S2:50%	90	90.26	100.28	S.D=1.97
S3:50%	90	89.12	90.02	%RSD=1.9
S4:100%	180	180.34	100.18	Mean=100.23
S5:100%	180	180.17	100.09	S.D=0.17
S6:100%	180	180.77	100.42	%RSD=1.7
S7:150%	270	270.11	100.04	Mean=100.12
S8:150%	270	270.30	100.11	S.D=0.09
S9:150%	270	270.63	100.23	%RSD=0.1

Robustness

Robustness of the proposed analytical method is a measure of its capacity to remain unaffected, and it reflects the reliability of the analysis with respect to deliberate changes in the parameters such as flow rate (1.0 ± 0.2 mL), column temperature (30 ± 5°C), mobile phase ratio of the mobile phase. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

CONCLUSION

RP-HPLC method for the simultaneous estimation of Aspirin and Ticlopidine in their combine dosage form was established and validated as per the ICH guidelines. Linearity was achieved for Aspirin and Ticlopidine in the range of 20-100µg/ml for Aspirin and 60-300µg/ml for Ticlopidine with correlation coefficients (r^2=0.999). The percentage recoveries of Aspirin and Ticlopidine were achieved in the range of 96.92-100.35%. which was with in the acceptance criteria. The percentage RSD was NMT 2% which proved the precision of the developed method. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust.

REFERENCES

1. British Pharmacopoeia, volume-2, London: The stationary office, 2004; Pg no-176, 1927.
2. Indian Pharmacopoeia. Indian Pharmacopoeia Commission. Ghaziabad: 2007; volume- 2: pg no257,745.
3. The Merck Index – An Encyclopedia of chemicals, Drugs, and biological, 13th edition, Merck Research Laboratories, Whitehouse station. New Jersey: 2001; pg no.856, 9506.
4. Grudeep R. Chatwal, Sham K. Anand. Instrumental methods of chemical analysis. Himalaya Publishing House, page no. 2.149-2.184.
5. Beckett A.H, Stnlake J.B, Practical pharmaceutical chemistry, 4th edition, part 2: CBS Publishers & Distributors; Delhi, 2001. Pg no. 284, 285, 296.
6. Gachet C: The platelet P2 receptors as molecular targets for old and new antiplatelet drugs. Pharmacol Ther., 2005 Nov; 108(2): 180-92. Epub 2005 Jun 13.Pubmed.
7. Shraddha C Gujarathi, Aditi R Shah, Swati C. Jagdale, Prasanna A. Datar, Vishnu P. Choudhari. Spectrophotometric Simultaneous Determination of Aspirin and Ticlopidine in Combined Tablet Dosage Form by First order Derivative Spectroscopy, Area under Curve (AUC) and Ratio Derivative

Spectrophotometric Methods. Int. J. Ph. Sci Review and Research, 2010; 3(1): 024.
8. RB Kakde, AG Barsagade, NK Chaudhary and DL Kale. Spectrophotometric Method for Estimation of Ticlopidine HCl in Bulk andDosage Form. Asian J. Research Chem., 2(1): Jan.-Mar. 2009.
9. Nafiz O. Can, Goksel Altokka, Hassan Y, Aboul Enein, Determination of Ticlopidine in Pharmaceutical Tablets by Flow Analysis Using UV Detection, journal of Liquid Chromatogrphy and Related Technologies, 2008; 31: 329- 3218.
10. Mishra P, Archana Dolly, Simultanious Determination of Clopidogrel and Aspirin in phamaceutical dosage forms, Indian Journal of Pharmaceutical Sciences, 2006; 68: 365, 368.
11. Patel G.F., Vekariya N.R, Dholakiya R.B, Estimation of Aspirin and Atorvastatin calcium in combine dosage form using Derivative Spectrophotometric method, International Journal of Pharmaceutical Research, 2010; 2: 62-65.
12. Dal B L, Verga F, Marzo A, Ambrosoli L, Poli A. Determination of Ticlopidine in Human Plasma by High-Performance Liquid Chromatography and Ultraviolet Absorbance Detection. J Chromatogr B Biomed Appl, 1995; 665(2): 404-9.
13. Vijay Ram, Govind Kher, Kapil Dubal, Bhavesh Dodiya, Hitendra Joshi. Development and validation of a stability indicating UPLC method for determination of ticlopidine hydrochloride in its tablet formulation. Saudi Pharmaceutical Journal, 2011; 19: 159–164.
14. Suresh Kumar S., Latif D.Jamadar, Krishnamurthy Bhat, Prashant B.Musmade, S.G.Vasantharaju, N.Udupa. Analytical Method Development and Validation for Aspirin. Int. J. Chem Tech Res., 2010; 2(1): 389-99.
15. Najma Sultana, Muhammad S. Arayne, Kiran A. Ali, and Muhammad Nawaz. Simultaneous Determination of Clopidogrel and Aspirin by RPHPLC from Bulk Material and Dosage Formulations Using Multivariate Calibration Technique. Journal of Chromatographic Science, Vol. 49, February 2011.
16. Nikelly J. G., Gas Chromatographic Determination of Acetylsalicylic Acid, Analytical Chemistry, 1964; 36(12): 2248- 2250.
17. ICH (2002) ICH Guidance on analytical method validation. In: Proceedings of the international convention on quality for the pharmaceutical industry, Toronto, Canada, September, 2002.