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LC method development and validation of aspirin and clopidogrel in pure API'S and its pharmaceutical dosage forms

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ABSTRACT

A simple, sensitive and precise reverse phase high performance liquid chromatographic method has been developed for the estimation of Aspirin and Clopidogrel in pure sample and its pharmaceutical dosage form. The mobile phase consists of Phosphate buffer (0.02 M Potassium dihydrogen phosphates, pH-3 adjusted with ortho phosphoric acid): acetonitrile in the ratio of 65:35 v/v delivered at a flow rate of 1.0 ml / min and wavelength of detection at 229 nm. The retention times of Aspirin and Clopidogrel were 2.673 and 3.627min respectively. The developed method was validated according to ICH guidelines. The result indicates that the method was found to be simple, rapid, and accurate and can be adopted in routine analysis of Aspirin and Clopidogrel in Pure sample and its Pharmaceutical dosage forms.

Keywords: Aspirin,Clopidogrel, Validation, Liquid chromatography.

INTRODUCTION

Aspirin is chemically 2-(acetyloxy) benzoic acid Fig[1], it is an Analgesic, Antipyretic, Anti inflammatory agent and also it posses Anti platelet aggregation property. Clopidogrel is chemically methyl (2S)-2-(2-chlorophenyl)-2-{4H, 5H, 6H, 7H-thieno [3, 2-c] pyridin-5-yl} acetate Fig[2], it is used as Anti platelet inhibitor. Its combination widely used for in the prevention of arterial and venous thrombosis Literature survey reveals that these drugs can be estimated only by LC-MS/MS[18], Spectrophotometric method[19]and HPLC method have been reported by using different solvents and columns that shows not much predicted values in current scenario. Hence the present study describes a simple, sensitive, accurate and precise HPLC method for the estimation of Aspirin and Clopidogrel in Pure API'S and its pharmaceutical dosage forms.

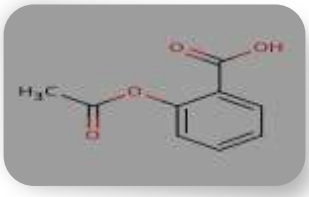

Fig.1 Structure of Aspirin

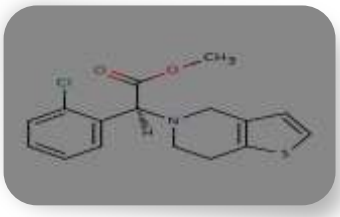

Fig.2 Structure of Clopidogrel

EXPERIMENTAL SECTION

Reagents and Chemicals

Aspirin and Clopidogrel API were obtained as gift sample from Senthan laboratories, Banglore. The branded formulation (tablets) (Asogrel-Atablets containing 75mg of Aspirin and 75mg of Clopidogrel) was procured from the local market. Acetonitrile, Methanol, Potassium di-hydrogen phosphate, Water and orthophosphoric acid used were of HPLC grade and purchased from Merck Specialities Private Limited, Mumbai, India.

Instrumetation

Chromatographic separation was performed on a Shimadzu chromatographic system((LC 20 AT VP) equipped with FID detector, Spin chrome (LC solutions) and Rheodyne injector (7725i) with 20µl fixed loop.

Chromatographic conditions

Devosil ODS Column, C_{18} (250 x 4.6mm, 5µ) was the column used for separation. Mobile phase consisting of a mixture of Buffer (0.02M Potassium dihydrogen phosphate, pH-3was adjusted with ortho phosphoric acid)and acetonitrile in the ratio 65:35 v/v was delivered at a flow rate of 1.0 ml/min with detection at 229 nm. The mobile phase was filtered through a 0.45 nylon filter and sonicated for 15 min. Analysis was performed at ambient temperature.

Pharmaceutical dosage form

Commercial tablets (Asogrel-A) were procured randomly from the local market.

Method development

Buffer (0.02M Potassium dihydrogen phosphate pH-3was adjusted with ortho phosphoric acid) and Acetonitrilein different proportions were tried and finally Buffer (0.02M Potassium dihydrogen phosphate, pH-3 was adjusted with ortho phosphoric acid) and Acetonitrile (65:35 v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable system suitability parameters. The chromatogram of working standard solution is shown in fig 3.

PROCEDURE

Preparation of mixed standard solution

Weigh accurately 37.6 mg of Clopidogrel and 37.8mg of Aspirin and transfer to 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase .Transfer 1.0 ml from the above stock solution into a 50 mL volumetric flask and make up the volume with mobile phase. Above stock solution contains 75 µg/ml of Clopidogrel and 75µg/ml of Aspirin. This solution is used for recording the chromatogram.

Procedure for analysis of tablets

20 tablets (each tablet contains 75mg of Clopidogrel and 75 mg of Aspirin) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Clopidogrel and Aspirin (75µg/ml) were prepared by dissolving weight equivalent to 75 mg of 75 mg of Aspirin and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 75µg/ml of Clopidogrel 75µg/ml of Aspirin was made by adding 1 ml of stock solution to 10 ml of mobile phase.shown in table.1

Calibration curve

Weigh accurately 37.8 mg of Aspirin and 37.8 mg of Clopidogrel and transfer to 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. Appropriate dilutions were made with mobile phase. Calibration curves were obtained by plotting the response (area of drug peak) versus concentration of drug. Regression equations were calculated. The method was found linear over a concentration range of 45µg/ml to 105µg/ml

METHOD VALIDATION

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre-analyzed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80%, 100%, 120%.The Accuracy data was given in Table 2 and 3.

Linearity

The method was linear in the range of 45µg/ml to 105µg/ml for Aspirin and Clopidogrel. Linear regression data was given in Table 4.The graphs shown in Fig 4 & 5.

Robustness

Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like Wavelength and flow rate variation. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed RP-HPLC method is robust. The result was shown in Table 5.

i) Effect of variation in Flow rate

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 0.8ml/min and1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 0.8ml/min and 1.2ml/min flow.Aspirin and Clopidogrel were resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min. From the above study it was established that the allowable variation in flow rates is 0.8ml/min and 1.2ml/min.

ii) Effect of variation of wavelength

A study was conducted to determine the effect of variation in wavelength. Standard solution prepared as per the test method was injected into the HPLC system at the wavelength of 229nm. The system suitability parameters were evaluated and found to be within the limits for the wavelength change of 2nm. Similarly sample solution was chromatographed.Aspirin and Clopidogrel were resolved from all other peaks and the retention times were comparable with those obtained. From the above study it was established that the allowable variation in wavelengths is 228nm and 230nm.Hence the method is robust.

Precision

The precision of the method was demonstrated. In the method presicionstudies, solutions of standard and sample were repeated thrice in a day and percent relative standard deviation (%RSD) for response factor was calculated. The method precision of %RSD of Aspirin was found to be 0.56. The method precision of %RSD for Clopidogrel was found to be 0.48. From the data obtained the developed RP-HPLC method was found to be precise.

RESULTS AND DISCUSSION

The proposed method was found to be linear in the concentration range of 45 to 105µg/ml for Aspirin and Clopidogrel. The method was specific since excipients in the formulation did not interfere in the estimation of Aspirin and Clopidogrel. Accuracy of the method was indicated by recovery values of Aspirin and Clopidogrel were 99.8% and 100.37%. Precision is reflected by %RSD values less than 2. These low values suggest sensitivity of the developed method. Validation parameters were summarized in Table 6.

CONCLUSION

The developed RP-HPLC method was simple, sensitive, precise and accurate and hence can be used in

routine for the determination of Aspirin and Clopidogrel in Pure API'S as well as in its pharmaceutical preparations.

Table 1 Analysis of tablet formulation (Asogrel-A)

Formulation	Analyte	Label claim (mg)	%label claim estimated*
	Aspirin	75	99.6
Tablet	Clopidogrel	75	98.8

*mean of six determinations

Table 2: Accuracy or Recovery of Aspirin

Recovery level	Amount taken (mcg/ml)	Accuracy Aspirin Area	Average area	Amount recovered (mcg/ml)	% Recovery	Average % Recovery
	75	915.963				
80%	75	998.025	927.25	75.12	100.16	
	75	867.762				
	90	1108.399				
100%	90	1126.391	1121.595	90.02	100.02	
	90	1130.003				99.8%
	105	1102.152				
120%	105	1222.264	1216.767	104.18	99.21	
	105	1325.885				

Table 3: Accuracy or Recovery of Clopidogrel

Recovery level	Amount taken (mcg/ml)	Accuracy Clopidogrel Area	Average area	Amount recovered (mcg/ml)	% Recovery	Average % Recovery
	75	1824.23				
80%	75	1830.66	1836.676	74.87	99.82	
	75	1855.137				
	90	2170.426				100.37%
100%	90	2324.729	2213.7	90.52	100.57	
	90	2146.015				
	105	2586.525				
120%	105	2676.139	2686.16	105.78	100.74	
	105	2795.826				

Table 4: Linear regression data for calibration curves

Parameter	Aspirin	Clopidogrel
Linearity range (µg/ml)	45-105	45-105
Correlation coefficient	0.9998	0.9962

Table 5: Robustness of Aspirin and Clopidogrel

Parameters	Optimum range	Conditions in procedure	Remarks
Flow rate (ml/min)	0.8-1.2	1.0	At lower flow rates the asymmetry factor was increased and at higher flow rates the relative retentions was decreased
Wavelength	228-230	229	Beyond the optimum range peak shape and symmetry was lost.

Table 6: Summary of validation parameters:

Parameter	Aspirin	Clopidogrel
Mean % recovery	99.8	100.37
Method Precision (%RSD)	0.56	0.48
Robustness	Robust	Robust
Retention time (min)	2.673	3.627
Theoretical plates	>2000	>2000
Tailing factor	<2	<2

Figure 3: **A Representative Chromatogram of Aspirin and Clopidogrel (Standard)**

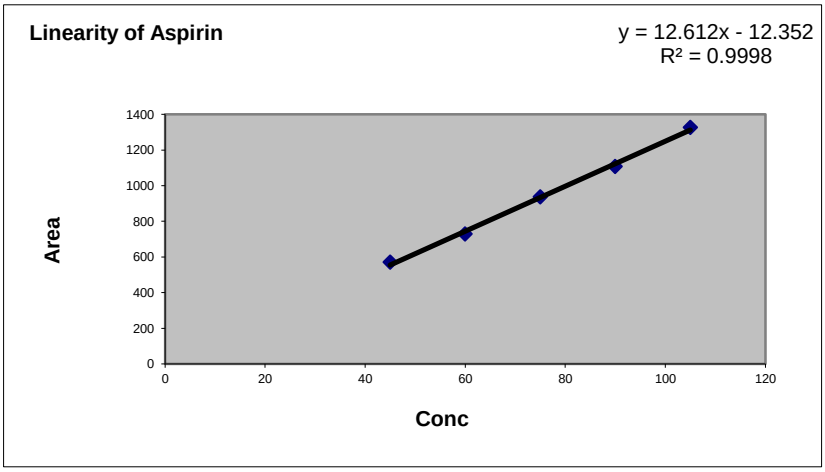

Figure 4: Linearity plot of Aspirin

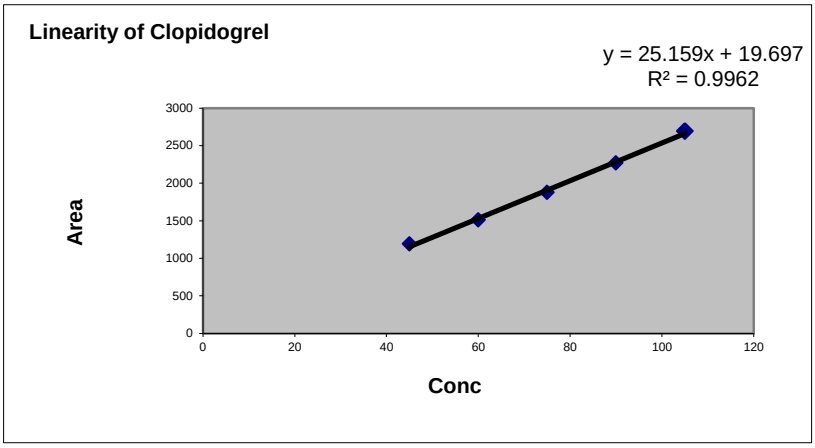

Figure 5: Linearity plot of Clopidogrel

REFERENCES

[1] B.K.Sharma, HPLC, Instrumental methods of chemical analysis,Goelpubkishers;24th edition;2005; p286-300.
[2] Gurudeep.R.Chatwal,Sharm.K.Anand,HPLC,Instrumental methods of chemical analysis;2010;p624-639.
[3] ICH, Text on Validation of Analytical Procedures, ICH – Q2A, International Conference on Harmonization, IFPMA, Geneva, 1995; 2-3: A–1 to A–3.
[4] ICH, Validation of Analytical Procedures Methodology, ICH – Q2B, International Conference on Harmonization, 1996; p1-3.
[5] ICH Guidelines, Q2 (R1) Validation of Analytical Procedures Text and Methodology, 2005; p1-6.
[6] British pharmacopoeia 2011 ,volume 1 ,page no 143-144
[7] United states pharmacopoeia 34 NF29 ,volume 2 part 1,page no. 1873-1875,1949-1951
[8] Indian pharmacopoeia -2010 volume-2,page no 806-807,849-850
[9] The Merck Index, An Encyclopedia Of Chemical, Drugs and Biologicals, Maryadele J.O. Neil.Eds,13th edition, Published by Merck Research Lab, Division of Merck and co. Inc., Whitehouse Station, NJ: 2006:148. NJ: 2006:86.
[10] SV Londhe**,** RS Deshmukh**,** SV Mulgund**,** KS Jain- Simultaneous determination of aspirin, atorvastatin calcium and clopidogrelbisulphate in capsules.Indian Journal of Pharmaceutical Sciences, 2011, 23-29.
[11] Sagar s panda- Simultaneous determination of Aspirin and Clopidogrelbisulphate in commercial formulation. Int.J. PharmTech, 2(1), 2010, 269-273.
[12] AnasRasheed, SumanPattanayak, T. Rama Rao- A specific, rapid, reliable and precise reversed phase ultra-performance liquid chromatographic method has been developed and validated for the simultaneous estimation of aspirin, clopidogrel and atorvastatin in tablet dosage form. Am. J. PharmTech Res.4 (4), 2014, 534-541.
[13] Prabhat K, Shrivastava**,** PawanBasniwal**,**DeeptiJainSushant ,K Shrivastava-Concurrent Estimation of Clopidogrel Bisulfate andAspirin in Tablets by Validated RP-HPLC Method. Indian Journal of Pharmaceutical Sciences, 2008, 667-669.

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