AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Analytical Method Development and Validation for the Simultaneous Estimation of Aspirin, Clopidogrel Bisulphate and Atorvastatin Calcium in Tablet Dosage Form

AnasRasheed*[1], SumanPattanayak[1], T. Rama Rao[1]

1. Department of Pharmaceutical Analysis,Avanthi Institute of Pharmaceutical Sciences, Guntapally, Hyderabad.

ABSTRACT

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. Chromatography was carried out on a 100 × 2.1 mm i.d., 1.7 µm C18 column with gradient flow programming mobile phase The mobile phase is a mixture of two solutions mobile phase A and mobile phase B in the ratio of 30:70. The mobile phase – A, contains buffer and methanol in the ratio of 93:7, the buffer used is 2.76 gms of Sodium phosphate in 100 ml. The mobile phase – B, contains 0.1% orthophosphoric acid and acetonitrile (60:40, v/v), at a flowrate of 0.5 mL/min. The detection was carried out by PDA detector. The retention times were about 0.55, 0.98 and 2.75 min for Aspirin, Clopidogrel and Atorvastatin, respectively. The runtime was 5 mins. The method was validated according to ICH guidelines and the acceptance criteria for accuracy, precision, linearity, specificity and system suitability were found to be under ICH limits. The method was linear in the range of 5-25 µg/mL of Aspirin, 5-25 µg/mL of Clopidogrel and 1-5 µg/mL of Atorvastatin. Limit of detection obtained were 0.02 µg/mL of Aspirin, 0.05 µg/mL of Clopidogrel and 0.07 µg/mL of Atorvastatin.

Keywords: Ultra performance liquid chromatography, Assay method, Gradient flow programming, tablet dosage forms, Aspirin, Clopidogrel bisulphate and Atorvastatin calcium.

*Corresponding Author Email: kmehmoodi@yahoo.in

Received 20 July 2014, Accepted 25 July 2014

Please cite this article as: Rasheed A *et al.*, Analytical Method Development and Validation for the Simultaneous Estimation of Aspirin, Clopidogrel Bisulphate and Atorvastatin Calcium in Tablet Dosage Form. American Journal of PharmTech Research 2014.

INTRODUCTION

Aspirin is 2-acetyloxybenzoic acid, used as an analgesic, antipyretic, anti-inflammatry and an antiplatelet. Aspirin's ability to suppress the production of prostaglandins and thromboxanes is due to its irreversible inactivation of the cyclooxygenase enzyme required for prostaglandin and thromboxane synthesis.(Shown in Figure.1)

Clopidogrel bisulphate is methyl (s)-2-chlorophenyl (4,5,6,7-tetrahydrothioeno-[3,2-C] pyridine-5-yl) acetate bisulphate, an oral, thienopyridine-class. It is used as an anti platelet agent. (Shown in Figure.2)

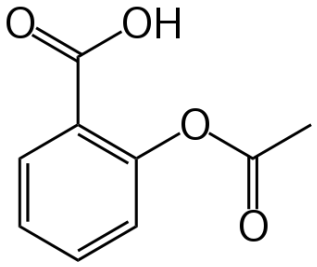

Figure 1: Molecular Structure of Aspirin

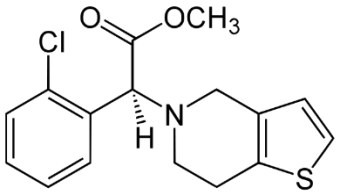

Figure 2: Molecular Structure of Clopidogrel

Atorvastatin calcium is [R-(R*,R*)]-2-(4-fluorophenyl)-β,ö-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1- heptanoic acid, a synthetic lipid-lowering agent. Atorvastatin is a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA)reductase. (Shown in Fig.3)

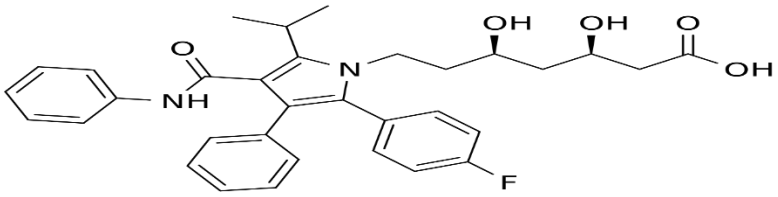

Figure 3: Molecular Structure of Atorvastatin

A detailed literature revealed that several analytical methods have been reported for the determination of Aspirin , Clopidogrel and Atorvastatin in pharmaceutical dosage forms.In our present knowledge, there is no method reported for the estimation of ASP, CLP and ATV in combination for tablet dosage form. Hence, the aim was to develop a specific, rapid, sensitive, and

accurate UPLC method which can estimate the three components simultaneously. The present investigation describes a specific, sensitive and rapid method for the simultaneous estimation of Aspirin, Clopidogrel and Atorvastatin in tablet dosage form. The present work aims with the developed method, only this mobile phase is sufficient for quantification of Aspirin , Clopidogrel and Atorvastatin either in combination for tablet dosage form.

MATERIALS AND METHODS

Materials:

Aspirin was donated by AurobindoPharma, Hyderabad. Clopidogrel and Atorvastatin were generously gifted by Hetero Drugs, Ltd; Hyderabad. Acetonitrile (HPLC grade) was purchased from Qualigens fine chemicals, Mumbai, India. Distilled, 0.45 µm filtered water used for HPLC and UPLC analysis and preparation of buffer. Buffers and all other chemicals were analytical grade.

Instrumentation:

An Acquity, Waters UPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2996 wavelength absorbance detector (PDA) was employed throughout the analysis. The data was acquired using Empower 2 software. The column used was Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm). A Band line sonerexsonicator was used for enhancing dissolution of the compounds. A Digisum DI 707 digital pH meter was used for pH adjustment. The mobile phase is a mixture of two solutions mobile phase A and mobile phase B in the ratio of 30:70. The mobile phase – A, contains buffer and methanol in the ratio of 93:7, the buffer used is 2.76 gms of Sodium phosphate in 100 ml. The mobile phase – B, contains 0.1% orthophosphoric acid and acetonitrile (60:40, v/v) with gradient flow programming was used as mobile phase at 0.5 mL/min. The column was maintained at ambient temperature.

Chromatographic Conditions:

The chromatographic elution was carried out in gradient mode using a mobile phase consisting of mixture of two solutions, mobile phase A and mobile phase B in the ratio of 30:70 v/v. Mobile Phase A (contains buffer and methanol in the ratio of 93:7, the buffer used is 2.76 gms of Sodium phosphate in 100 ml). Mobile Phase B (contains 0.1% orthophosphoric acid and acetonitrile (60:40, v/v)). The analysis was performed at ambient temperature using a flow rate of 0.5 mL/min with a run time of 5 mins. The eluent was monitored using PDA detector. The mobile phase was filtered through 0.45 µm micron filter prior to use.

Preparation of Standard Stock Solution

Accurately weighed Aspirin (150 mg), Clopidogrel (75mg) and Atorvastatin (20 mg) were transferred to 100 mL volumetric flasks, dissolved and diluted to the mark with methanol to obtain a standard stock solution of Aspirin (100 µg/mL), Clopidogrel (100 µg/mL) and Atorvastatin (50µg/mL). From the prepared stock solution 1 mL solution was transferred to a 25 mL volumetric flask, and diluted to the mark with mobile phase to obtain a mixed working standard solution of Aspirin (15 µg/mL), Clopidogrel (15 µg/mL) and Atorvastatin (5 µg/mL).

Preparation of Sample Solution

The powdered 20 tablets (Ecosprin Gold Forte), each containing 150 mg Aspirin, 75 mg Clopidogrel and 20 mg Atorvastatin, were weighed and analyzed: a quantity of powder equivalent to one capsule was weighed and transferred to a 100 mL volumetric flask containing 50 mL methanol and sonicated for 15 min. The flask was allowed to cool down to room temperature and the volume was made up to the mark with methanol to obtain sample stock solution of Aspirin (100 µg/mL), Clopidogrel (100 µg/mL) and Atorvastatin (50 µg/mL). The solution was filtered using a 0.45 µm micron filter. From the prepared stock solution 1 mL solution was transferred to a 25 mL volumetric flask and diluted to the mark with mobile phase to obtain a working sample solution of ASP (15 µg/mL),CLP (15 µg/mL) and ATV (5 µg/mL).

Method Validation

The developed method was validated in terms of specificity, system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

Specificity Study

The specificity of the RP-UPLC method was checked by comparison of chromatograms obtained from standard, sample and the corresponding placebo.

Linearity and Range

The linearity of the method was determined at seven concentration levels ranging from 5-25 µg/mL for Aspirin, Clopidogrel and 1-5 µg/mL for Atorvastatin. The calibration curves were constructed by plotting peak areas versus concentration of Aspirin, Clopidogrel and Atorvastatin. The slope, Y-intercept and correlation coefficient were calculated. (Shown in Figure.4,5 and 6)

Accuracy (% Recovery)

The accuracy of the method was evaluated in triplicate at three concentration levels, 50, 100 and 150 % of the target test concentration (15 µg/mL of Aspirin, Clopidogrel, 5 µg/mL of Atorvastatin). The percentages of recoveries were calculated.

Precision

Precision was investigated using the sample preparation procedure for six real samples of marketed tablets (Ecosprin Gold Forte 20). Method Precision (Intra-day): The precision of the method was evaluated by carrying out six independent assays of (15 µg/mL of) Aspirin, (15 µg/mL of) Clopidogrel, (5 µg/mL of) Atorvastatin test samples against qualified reference standard. Six test samples were assayed against reference standard.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were estimated using signal - to - noise ratio of 3:1 and 10:1 as per ICH guidelines.

Robustness

The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions: Flow rate (±0.010), composition of mobile phase (53:47 and 57:43, v/v), column temperature (± 5°C) and wavelength of detection (± 2nm).

System-Suitability Test

The system suitability tests represent an integral part of the method and are used to ensure adequate performance of the chromatographic system. The parameters, retention time (RT), theoretical plates (N), tailing factor (T), peak asymmetry (As) and repeatability were evaluated using five replicate injections of the drugs at a concentration of Aspirin (15 µg/mL), Clopidogrel (15 µg/mL) and Atorvastatin (5 µg/mL).

RESULTS AND DISCUSSION

To develop a precise, linear, specific & suitable stability indicating RP-UPLC method for the simultaneous estimation of aspirin, clopidogrel and atorvastatin in tablet dosage form, different chromatographic conditions for its validation were applied & the results observed are presented (Shown in Table 1 and 2). Gradient elution is used, it decreases the retention of the later-eluting components so that they elute faster, giving narrower (and taller) peaks.(Show in Figure. 7). The results of Correlation coefficient (r) LOD, LOQ, Accuracy, Precision, Intraday, Interday, and Repeatability are shown in Table 1. The results of System Suitability Parameters consisting of Retention time, Theoretical plates, Asymmetry are shown in Table 2. The standard chromatogram of simultaneous estimation of aspirin, clopidogrel and atorvastatin in tablet dosage form is shown in figure. 7. The calibration curve of aspirin, clopidogrel and atorvastatin is shown in Figure 4,5 and 6 respectively.

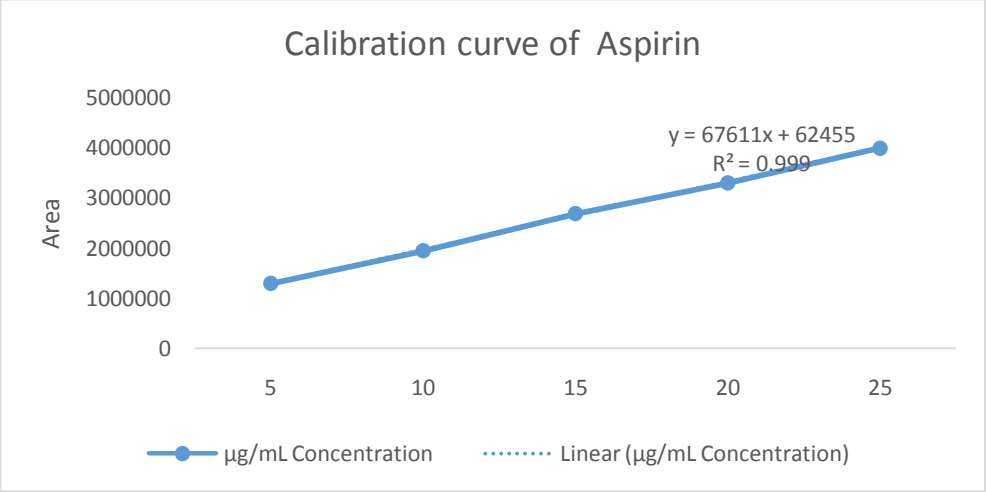

Figure 4: Calibration curve of Aspirin

For Clopidogrel

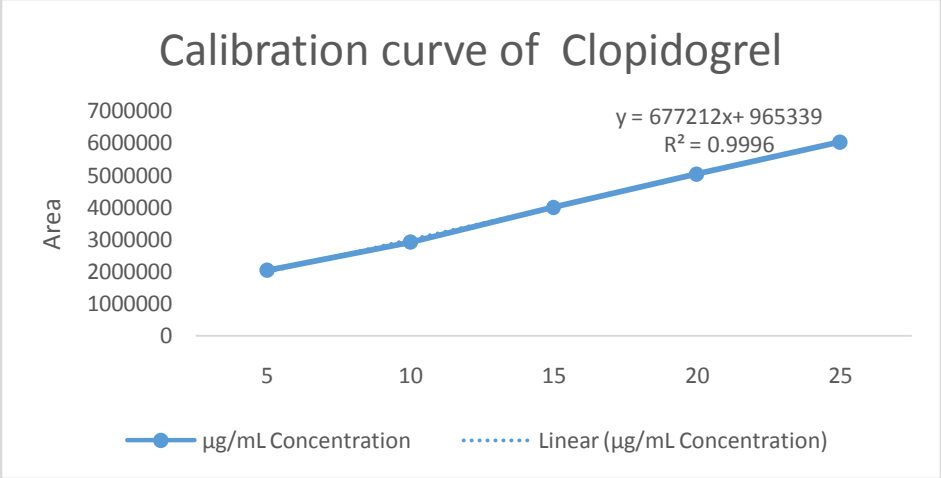

Figure 5: Calibration curve of Clopidogrel

For Atorvastatin

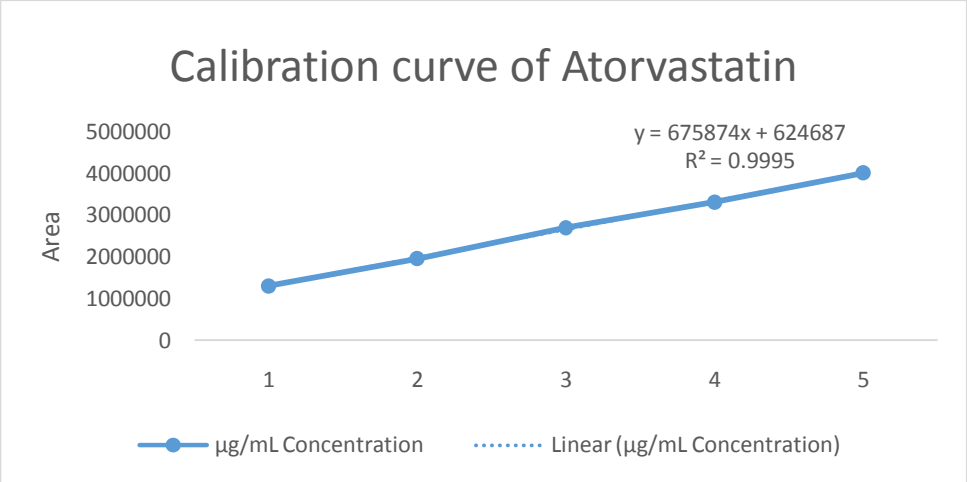

Figure 6: Calibration curve of Atorvastatin

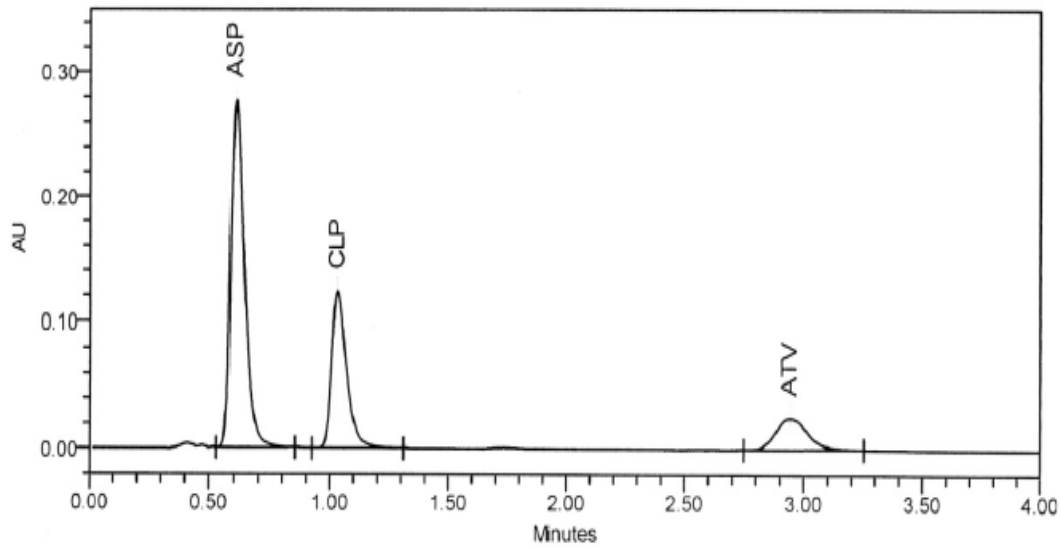

Figure 7: Chromatogram showing retention time of Aspirin (0.55 min), Clopidogrel (0.98 min) and Atorvastatin (2.75 min) simultaneously.

Table 1: Results from Analysis and Calibration Curves

Parameters	Aspirin	Clopidogrel	Atorvastatin
Correlation coefficient (r)	0.9994	0.9996	0.9995
LOD (µg/mL)	0.02	0.05	0.07
LOQ (µg/mL)	0.06	0.12	0.17
Accuracy (%) ± % RSD	99.63 ± 0.24	99.21 ± 0.45	99.34 ± 0.65
Precision (% assay ± % RSD)			
Intraday (n = 6)	100.31 ± 0.44	100.21 ± 0.24	100.35 ± 0.48
Interday (n = 6)	100.06 ± 0.54	99.96 ± 0.62	100.26 ± 0.56
Repeatability (% RSD)	0.089	0.102	0.234

System Suitability Parameters

Table 2: Results of System Suitability Parameters

Parameters	Aspirin	Clopidogrel	Atorvastatin
Retention time (min) ± % RSD	0.55 ± 0.06	0.98 ± 0.05	2.75 ± 0.05
Theoretical plates ± % RSD	2327.36 ± 0.50	3958.87 ± 0.40	6347.51 ± 0.30
Asymmetry ± % RSD	1.08 ± 0.05	1.25 ± 0.06	1.05 ± 0.12

CONCLUSION

A specific RP-UPLC method has been developed & validated for the simultaneous estimation of aspirin, clopidogrel and atorvastatin in tablet dosage form. Further the proposed RP-UPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is suitable for assay studies which can help in the analysis of aspirin, clopidogrel and atorvastatin in combination for tablet dosage form with the developed method, only this mobile phase is sufficient for their quantification.

REFERENCES:

1. David A. Williams, William O. Foye, Thomas L. Lemke; Foye's principles of medicinal chemistry; 6th Ed (2008) Wolters Kluwer Health; 698-728.
2. Tripathi KD; Essentials of Medical Pharmacology; 6th Ed (2008)Jaypee Brothers Medical Publishers(P)Ltd; 561-574.
3. R. Vani, B. Vijaya Kumar, AnasRasheed; Analytical Development and Formulation of Ramipril and Hydrochlorothiazide In Combination With Selective Excipients, R. Vani, et al. Int J Pharm 2013; 3(2): 348-35.
4. Vani R and Rasheed A: Formulation and evaluation of Hydrochlorothiazide and Ramipril Mouth Dissolving Tablet using different Superdisintegrants. Int J Pharm Sci Res 2014; 5(1): 207-12.doi: 10.13040/IJPSR. 0975-8232.5(1).207-12.
5. Rasheed A and Farhat R: Combinatorial Chemistry. Int J Pharm Sci Res 2013: 4(7); 2502-2516. doi: 10.13040/IJPSR. 0975-8232.4(7).2502-16.
6. Rasheed A, Fathima I and Altaf M: Attention Deficit Hyperactivity Disorder (ADHD): An Overview. Int J Pharm Sci Res 2013; 4(4); 1669-1683.
7. Vani R. et al., Validation and Derivative Spectroscopy of PrasugrelHClIn Bulk and Formulation. American Journal of PharmTech Research 2013.
8. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of analytical procedures: definitions and terminology, Geneva (1996).
9. Rasheed A and QasimMd: A Review of Natural Steroids and their Applications. Int J Pharm Sci Res. 2013; 4(2); 520-531.