Research Article

A Validated RP-HPLC Method for Simultaneous Estimation of Aspirin and Rosuvastatin in Tablet Dosage Form

Kumaraswamy.Gandla[1]*, R. Lalitha[1], Konam Kishore[2] and Rakam Gopikrishna[3]

[1]Department of Pharmaceutical Analysis, Teegala Ram Reddy College of Pharmacy, Meerpet, Hyderabad- 505172, Telangana, India.

[2]Holy Mary Institute of Technology and Science College of Pharmacy, Bogaram, Hyderabad, Telangana, India.

[3]Chaitanya College of Pharmacy Education & Research, Hanmakonda, Telangana, India.

ABSTRACT

An isocratic separation was carried out using X-Terra (4.6 ×150mm, 5µm particle size) column and Methanol: Buffer45:55%v/v) as mobile phase with quantification carried out at a wavelength of 215nm. The retention time of the Aspirin, Rosuvastatin was 3.234, 1.694 minutes, respectively with theoretical plate count and asymmetry as per the ICH limits. The % assay of Aspirin and Rosuvastatin were 99.5% and 98.99%.The flow rate was found to be 1ml/min. The linear regression analysis data for the calibration plots showed a good linear relationship for Aspirin and Rosuvastatin over a concentration range of 5-25µg/ml and 6.25-31.25 with correlation co-efficient of 0.999 for Aspirin and 0.999 for Rosuvastatin. The limits of detection and quantitation were found to be 1.08, 0.80 and 3.27, 2.45 µg/ml respectively.

Keywords: Aspirin, Rosuvastatin, PDA Detector, RP-HPLC.

INTRODUCTION

Rosuvastatin[1] is chemically 3-ethyl 5-methyl (4*RS*)-2-[(2-aminoethoxy)methyl]-4-(2chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate (fig.1) belongs to the class of Calcium channel blocker, used as anti-anginal. Solubility - Slightly soluble in water and in isopropyl alcohol, sparingly soluble in dehydrated alcohol, freely soluble in methanol.

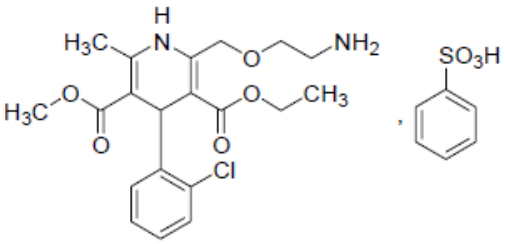

Fig. 1: Chemical Structure of Rosuvastatin

Aspirin Tablets contain the tert-butylamine salt of perindopril, the ethyl ester of a non-sulfhydryl angiotensin-converting enzyme (ACE) inhibitor. Aspirin is chemically described as (2S,3∝S,7∝S)-1-[(S)-N-[(S)-1-Carboxy-butyl] alanyl]hexahydro-2-indolinecarboxylic acid, 1-ethyl ester, compound with tert-butylamine (1:1). Its molecular formula is ($C_{23}H_{43}N_3O_5$).

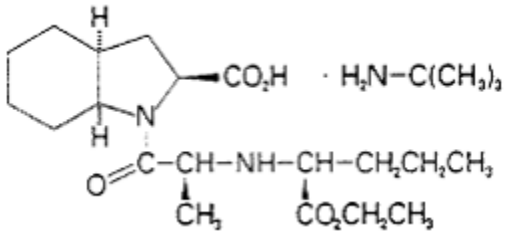

Fig. 2: Chemical Structure of Aspirin

Aspirin is a white, crystalline powder with a molecular weight of 368.47 (free acid) or 441.61 (salt form). It is freely soluble in water (60% w/w), alcohol and chloroform. Perindopril is the free acid form of Aspirin, is a pro-drug and metabolized in vivo by hydrolysis of the ester group to form perindoprilat, the biologically active metabolite.

From the literature survey it was found that many methods are available for determination of Aspirin and Rosuvastatin individually and few methods in combination with other drugs.
In the proposed study an attempt will be made to develop a s HPLC method for simultaneous estimation of Aspirin and Rosuvastatin in pharmaceutical formulation (tablet).
Pharmaceutical grade of Rosuvastatin, and Aspirin were kindly supplied as gift samples by Sura Labs pvt Ltd, Dislhuknagar, Hyderabada certified to contain > 99% (w/w) on dried basis. Commercially available *Coversyl-AM* Serdia Pharmaceuticals (India) Ltd, Mumbai, patalganga, India) tablets claimed to contain 5 mg Rosuvastatin and 4 mg Aspirin have been utilized in the present work. All chemicals and reagents used were of HPLC grade and were purchased from Agenta Chemicals, Hyderabad, India.

Chromatographic system and conditions:
The HPLC system waters 2695 consisted of Quaternary pump. The Analytical column and Isocratic elution with X-Terra (4.6 ×150mm, 5µm particle size) column and Methanol: Buffer45:55%v/v) as mobile phase with quantification carried out at a wavelength of 215 nm. Before analysis the mobile phase was filtered through a (0.45 µm) membrane and degassed by **ultrasonification**. And injection volume was 10µl. All the experiments were performed at ambient temperature.
Pharmaceutical grade of Rosuvastatin and Aspirin were kindly supplied as gift samples by Sura Labs Pvt. Ltd., Hyderabad, certified to contain > 99% (w/w) on dried basis. Commercially tablets claimed to contain 5 mg Rosuvastatin and 4 mg Asprinhave been utilized in the present work. All chemicals and reagents used were of HPLC grade and were purchased from Agenta Chemicals, India.

Standard solutions and calibration graphs for chromatographic measurement:
Stock standard solutions were prepared by dissolving separately 10 mg of Aspirin and Rosuvastatin in 10 ml mobile phase (1000 µg/ml). The standard calibration solutions were prepared by appropriate dilution of the stock solution with Methanol: Buffer45:55%v/v)) to reach a concentration range of 5-25µg/ml and 6.25-31.25 µg/ml. for Rosuvastatin and Aspirin. 10 µl injections were made for each concentration and chromatographed under the optimized conditions described above. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

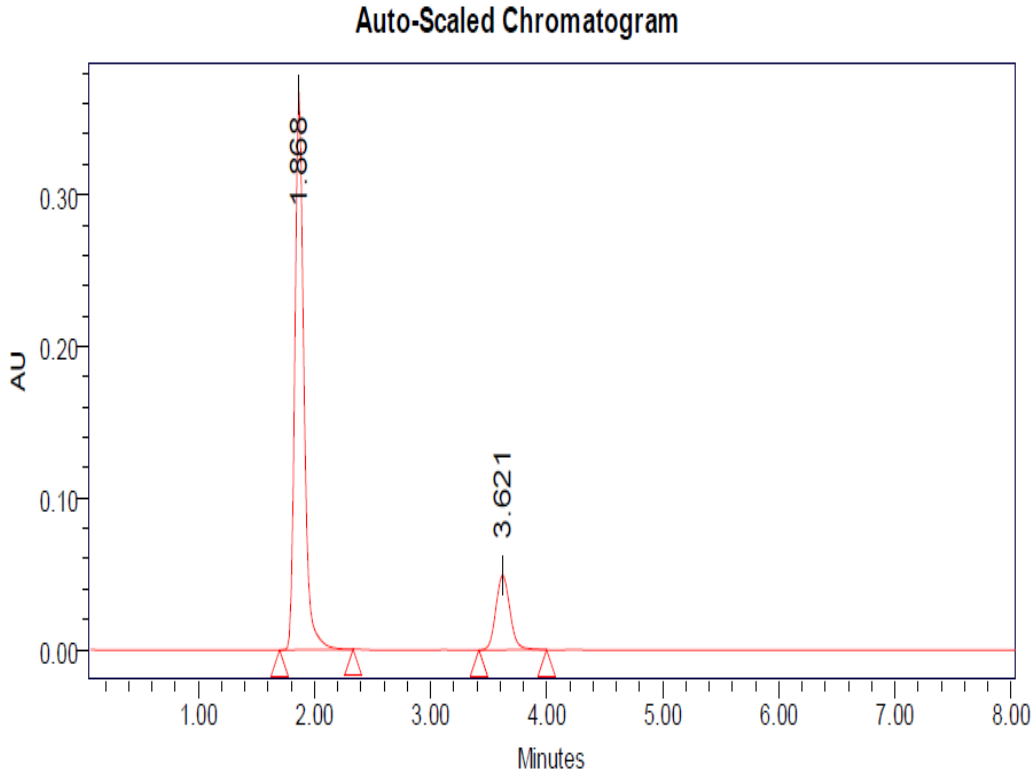

Fig. 3: Typical Chromatogram for Aspirin and Rosuvastatin

Preparation of Sample Solution

Take average weight of two Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Aspirin, Rosuvastatin sample into a 10ml clean dry volumetric flask and add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Procedure

Further pipette 0.186 ml of Aspirin, Rosuvastatin from above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Method validation

The developed method was validated according to the ICH guidelines. The system suitability was evaluated by six replicate analyses of Rosuvastatin and Aspirin mixture at a concentration of 50 µg/ml Aspirin and 25 µg/ml Rosuvastatin. The acceptance criteria were a R.S.D. of peak areas and retention times less than 2%, Theoretical plate numbers (N) at least 2500 for each peak and tailing factors (T) less than 1% for Aspirin and Rosuvastatin.

Standard calibration curves were prepared in the mobile phase with six concentrations ranging from 5-25µg/ml and 6.25-31.25 µg/ml for Aspirin and Rosuvastatin into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. To study the reliability and suitability of the developed method, recovery experiments were carried out at three levels 50, 100 and 150%. Known concentrations of commercial tablets were spiked with known amounts of Asprin and Rosuvastatin. At each level of the amount six determinations were performed and the results obtained were compared with expected results. Recovery for pharmaceutical formulations should be within the range 100±5%. The percent R.S.D. of individual measurements was also determined. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) for 2 consecutive days. Three different concentrations of Asprin and Rosuvastatin were analyzed in six independent series in the same day (intra-day precision) and 3 consecutive days (inter-day precision).. The repeatability of sample application and measurement of peak area for active compounds were expressed in terms of percent RSD.

System suitability parameters

Table 1: System Suitability Parameters

S. No.	Parameter	Rosuvastatin	Aspirin
1.	Retention Time (min)	1.694	3.234
2.	Theoretical Plates	6993	5735
3.	Tailing factor	1.23	1.12
4.	Area	1429524	300414
5.	Resolution	10.69	

The system suitability parameters were found to be within the specified limits for the proposed method.

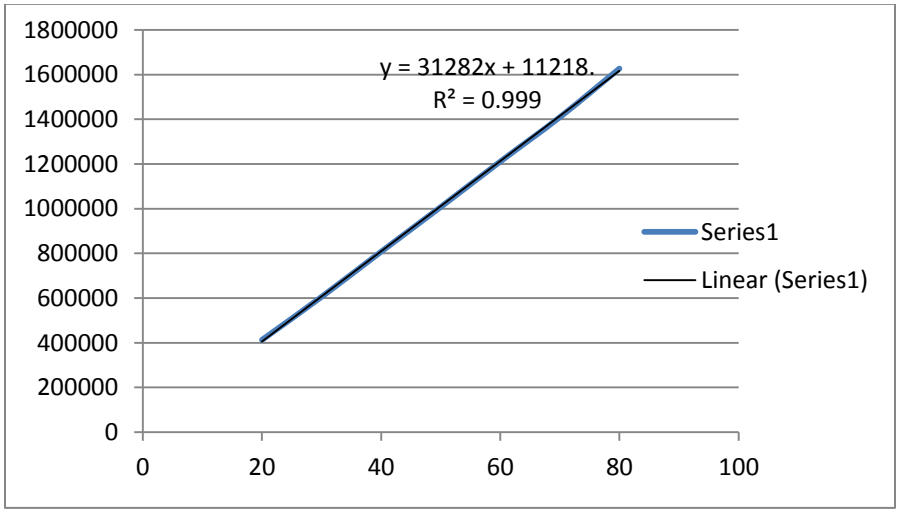

Fig. 4: Linearity Plot Aspirin

Data of Linearity (Rosuvastatin)

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	20193
20	412977	y-Intercept	1902
30	605369	Correlation Coefficient	0.999
40	807564		
50	1007428		
60	1210925		
70	1409560		
80	1627087		

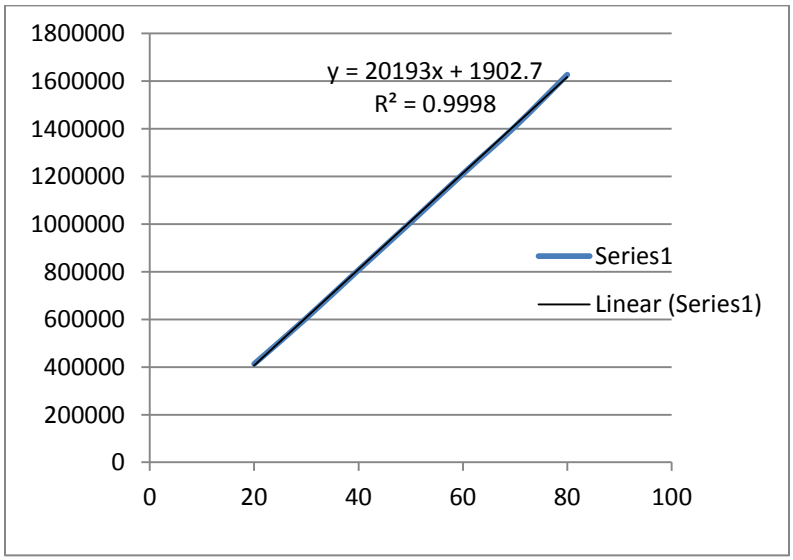

Fig. 5: Linearity Plot of Rosuvastatin

The linearity range was found to be 50-150 µg/ml for both Rosuvastatin and Aspirin. Calibration curve was plotted and correlated Co-efficient for both the drugs found to be 0.999.

LIMIT OF DETECTION (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × S.D / Slope

Table 5: LOD Results of the method

Drug	Amount(µg/ml)
Rosuvastatin	0.80
Aspirin	1.08

From the above, the LOD values of Rosuvastatin and Aspirin were found to be 0.80 and 1.081 µg/ml respectively.

LIMIT OF QUANTITATION (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10× S.D / Slope

Table 6: LOQ Results of the Method

Drug	Amount(µg/ml)
Rosuvastatin	2.45
Aspirin	3.27

From the above, the LOQ values of Rosuvastatin and Aspirin were found to be 2.45 and 3.27 µg/ml respectively.

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic

phase to less organic phase ratio for Aspirin, Rosuvastatin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±10%. The standard and samples of Aspirin, Rosuvastatin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

All chromatograms were examined to determine if compounds of interest co-eluted with each other or with any additional excipients peaks. Marketed formulations were analyzed to determine the specificity of the optimized method in the presence of common tablet excipients. Limit of detection (LOD) and limit of quantitation (LOQ) were estimated from the signal-to-noise ratio. LOD and LOQ were calculated using 3.3σ/s and 10σ/s formulae, respectively, where, σ is the standard deviation of the peak areas and *s* is the slope of the corresponding calibration curve. To evaluate robustness of HPLC method a few parameters were deliberately varied. The parameters included variation of flow rate.

RESULTS AND DISCUSSION

During the optimization of HPLC method, columns (X-Bridge (4.6 ×150mm, 5µm particle size), Symmetry (4.6 ×150mm, 5µm particle size),X-Terra (4.6 ×150mm, 5µm particle size), two organic solvents (acetonitrile,methanol and water Initially methanol:water, acetonitrile:water, The Rosuvastatin eluted with these Different ratios of various mobile phases were tried but Aspirin was retained. Then, with Methanol :Acetonitrile: :Water all the two drugs eluted. The mobile phase conditions were optimized so the peak from the first-eluting compound did not interfere with those from the solvent, excipients. Other criteria, *viz*. time required for analysis, appropriate k range (1<k<10) for eluted peaks, assay sensitivity, solvent noise were also considered. Finally a mobile phase consisting of a mixture of Methanol: Buffer45:55%v/v) were selected as mobile phase to achieve maximum separation and sensitivity. Flow rates between 0.8 to 1.3 ml/min were studied. A flow rate of 1.0 ml/min gave an optimal signal to noise ratio with a reasonable separation time. Using a reversed phase C18 (X-Terra (4.6 ×150mm, 5µm particle size) column, the retention times for Rosuvastatin and Aspirin were observed to be 1.694 and 3.234 min. respectively. Total time of analysis was less than 10 min. The chromatogram at 215 nm showed a complete resolution of all peaks.

Representative chromatograms of standard solutions (a) standard solution of Aspirin (50 µg/ml); (b) standard solution of Rosuvastatin (25 µg/ml) and (c) a standard solution containing 50 µg/ml Aspirin, 25 µg/ml Rosuvastatin.

Validity of the analytical procedure as well as the resolution between different peaks of interest is ensured by the system suitability test. All critical parameters tested met the acceptance criteria on all days. As shown in the chromatogram, all three analytes are eluted by forming symmetrical single peaks well separated from the solvent front. Excellent linearity was obtained for all the two drugs in the range of 5-25µg/ml and 6.25-31.25 for Asprin and Rosuvastatin. The correlation coefficients (r^2) were found to be greater than 0.999 (n=6) in all instances. The results of calibration studies are summarized in Table 1. The proposed method afforded high recoveries for Asprin and Rosuvastatin tablet. Results obtained from recovery studies presented in Table 2, indicate that this assay procedure can be used for routine quality control analysis of this ternary mixture in tablet. Precision of the analytical method was found to be reliable based on % RSD (< 2%) corresponding to the peak areas and retention times. The % RSD values were less than 2, for intra-day and inter-day precision. Hence, the method was found to be precise for all the two drugs.

The chromatograms were checked for the appearance of any extra peaks. It was observed that single peak for Rosuvastatin 1.694 and Aspirin, 3.234 were obtained under optimized conditions, showing no interference from common tablet excipients and impurities. Also the peak areas were compared with the standard and % purity calculated was found to be within the limits. These results demonstrate the specificity of the method

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