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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND ASPIRIN IN MARKETED FORMULATION

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ABSTRACT

A simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method has been developed for the simultaneous determination of Rosuvastatin calcium and Aspirin from Capsule dosage forms using C18 column (Grace Smart (250mm x 4.6mm, 5 µm). The sample was analyzed using Water (0.5 ml tri-ethyl amine in 100mL of double distilled water): Acetonitrile in the ratio of 50:50(% v/v) pH adjusted to 4.0 with orthophosphoric acid, as a mobile phase at a flow rate of 1.0 mL/min and detection at 243 nm. The retention time for Rosuvastatin calcium and Aspirin was found to be 4.30 and 3.44 min respectively. The method can be used for estimation of combination of these drugs in capsules. The method was validated as per ICH guidelines. The linearity of developed method was achieved in the range of 6 – 14 µg/mL (r^2=0.9997) for Rosuvastatin calcium and 45 – 105 µg/mL (r^2=0.9989) for Aspirin and assay of capsules were between 98.0-102.0%. Due to these attributes, the proposed method could be used for routine quality control analysis of these drugs in combined dosage forms.

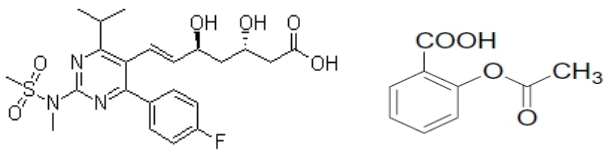
KEYWORDS: RP-HPLC Method, RSV and ASP, Rosuvastatin and Aspirin.

INTRODUCTION

Rosuvastatin Calcium is official in Indian pharmacopoeia. It is chemically (E)-(3R,5S)-7-{4 - (4-fluorophenyl)-6-isopropyl-2-{methyl(methylsulphonyl amino)] pyrimidin -5-yl}-3,5-dihydroxyhepten-6-oic acid calcium. It is used as a lipid lowering agent, act by inhibition of 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA) reductase. This enzyme catalyses the conversion of HMG-CoA to mevalonate, an early and rate limiting step in cholesterol biosynthesis[1]. Aspirin (ASP) is chemically 2-acetoxybenzoic acid and used as an analgesic, antipyretic, anti-inflammatory and anti-thrombic agent[1,2]. Structures of Aspirin and Rosuvastatatin Calcium are shown in figure 1.

Combined dosage forms of RSV and ASP are available in the market. Clinical trials showed that combination therapy when used in dyslipidaemic patient with coronary heart diseases reduced cardiovascular revents.

A literature survey regarding quantitative analysis of these drugs revealed that there were several analytical methods for RSV like extractive spectrophotometry[4], HPLC[5] and HPTLC[6]. ASP is official in Indian pharmacopoeia and titrimetry[2] method is official method for its assay. Also HPLC[7] method has been reported for its estimation. This paper describes RP-HPLC method for the simultaneous estimation of RSV and ASP in combination in capsule dosage form.



Rosuvastatin Aspirin

Figure: 1 Structure of Rosuvastatin and Aspirin.

MATERIALS AND METHODS

Materials

Working standards of pharmaceutical grade rosuvastatin and aspirin were obtained as generous gifts from Cadila pharmaceuticals. It was used without further purification. Fixed dose combination capsule (Unistar) containing 10 mg rosuvastatin calcium and 75 mg aspirin were procured from local market. All chemicals and reagents of analytical grade were purchased from s d Fine-chem. Ltd.

Preparations of mobile phase and stock solution:

100 ml of double distill water was taken and 0.5 ml of tri-ethyl amine was added .This solution was mixed with 100 ml of Acetonitrile. Finally the pH was adjusted to 4.0 with ortho phosphoric acid. The solution was sonicated for 10 minutes and filtered using Whatman filter paper (No.1). RSV and ASP were weighed (25 mg each) and transferred to two separate 25 ml volumetric flasks and dissolved in methanol, which gives 1000 µg/ ml of RSV and ASP, respectively. For RSV 5.0 ml was further diluted to 50 ml with methanol to obtained the final concentration of 100 µg/ ml from this working solution of RSV (6,8,10,12,14 µg/ml) were prepared. For ASP 15 ml was further diluted to 100 ml with methanol to obtained the final concentration of 150 µg/ ml from this solution working solution of ASP (45,60,75,90,105 µg/ml) were prepared.

Chromatographic conditions:

A reverse phase C18 column equilibrated with mobile phase water: Acetonitril (50:50) (adjusted to pH 4.0 with the orthophosphoric acid) was used. Mobile phase flow rate was maintained at 1.0 ml/min and effluents were monitored at 243 nm. The sample was injected using a 20 µL fixed loop, and the total run time was 6 min. Calibration curves were constructed by plotting average peak area versus concentrations and regression equations were computed for RSV and ASP.

Determination of RSV and ASP in their combined dosage forms:

The content of twenty capsules were taken and weighed. Powder equivalent to RSV 10.0 mg or 75.0 mg ASP was accurately weighed and transferred to a 100 ml volumetric flask, 20 ml of methanol was added and sonicated for 5 min then volume was adjusted up to the mark with methanol. The above solution was filtered using Whatman filter paper No.1. Appropriate volume of the aliquot was transferred to a 50 ml volumetric flask and the volume was made up to the mark with mobile phase to obtain 10 µg/ ml of RSV and 75 µg/ml of ASP. The solution was injected at above chromatographic conditions(n=6) and peak areas were measured. The method

was validated for accuracy, precision, specificity, detection limit, quantitation limit and robustness.

Accuracy[9]:

The accuracy of the method was determined by calculating recoveries of RSV and ASP by method of standard additions. Known amount of RSV (0, 4, 6, 8 µg/ml) and ASP (0, 15, 30,45 µg/ml) were added to a pre-quantified sample solution, and the amount of RSV and ASP were estimated.

Precision[9]:

The intra day and inter day precision study of RSV and ASP was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days (first, second and third day) for 3 different concentrations of RSV (8, 10, 12 µg/ml) and ASP (60,75,90 µg/ml), and the results are reported in terms of relative standard deviation (RSD), [Table - 2]. The Repeatability studies were carried out by estimating response of one test concentration concentrations (10+75 µg/ml) for 6 times and results are reported in terms of relative standard deviation (RSD).

Detection limit and Quantitation limit[9]:

A calibration curves (n=5) were prepared using concentrations in the range of 6-14 µg/ml for RSV and 45-105 µg/ml for ASP. The standard deviation of y-intercepts of regression lines were determined and kept in following equation for the determination of detection limit and quantitation limit. Detection limit= 3.3σ /s; quantitation limit= 10σ/s; where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve.

Robustness:

Robustness of the method was studied by changing the composition of organic phase by ±5.0% and the pH by ±0.2, and also by observing the stability of the drugs for 24 h at 35 ^{o} temperature in the mobile phase.

RESULTS AND DISCUSSION

Optimization of mobile phase was performed based on resolution, asymmetric factor and peak area obtained for both RSV and ASP. The mobile phase Water (0.5 ml tri-ethyl amine in 100ml) : Acetonitrile (50 : 50) adjusted to pH 4 using ortho phosphoric acid was found to be satisfactory and gave two symmetric and well-resolved peaks for RSV and ASP. The resolution between RSV and ASP was found to be 4.90, which indicates good separation of both the compounds. The retention time for RSV and ASP were 4.30 min and 3.44 min, respectively [Figure - 2]. The asymmetric factors for RSV and ASP were 1.25 and 1.37, respectively. Overlain UV spectra of both RSV and ASP showed that both the drugs absorbs appreciably at 243 nm so, 243 nm was selected as the detection wavelength in liquid chromatography.

The calibration curve for RSV was obtained by plotting the peak area of RSV versus the concentration of RSV over the range of 6-14 µg/ml, and it was found to be linear with r^2= 0.9997. Similarly, the calibration curve for ASP was obtained over the range of 45-105 µg/ml and was found to be linear with r^2= 0.9989. The data of regression analysis of the calibration curves are shown in [Table -1]. The detection limit for RSV and ASP were 0.50 µg/ml and 1.06 µg/ml, respectively. The quantitation limit for RSV and ASP were 1.58 µg/ml and 3.30 µg/ml, respectively. The validation parameters are summarized in [Table-2]. The recoveries of RSV and ASP were found to be in the range of 98.02-100.68% and 98.38-101.42%, respectively. The system suitability test parameters are shown in [Table-3]. The liquid chromatographic method was applied to the determination of RSV and ASP in their combined dosage forms. The results for RSV and ASP were comparable with the corresponding labeled amounts [Table-4]. Proposed study describes a new RP-HPLC method for the estimation of RSV and ASP combination in mixture using simple mobile phase. The method gives good resolution between both the compounds with a short analysis time (<8 min). The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of RSV and ASP in their combined dosage form.

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REFERENCES

1. Rang HP, Dale MM, Ritter JM, Flower RJ. Rang and Dale's Pharmacology, 6th Edn. Churchill Livingstone, Elsevier Science Ltd; 2007, p. 323-329.
2. Indian Pharmacopoeia**,** The Indian Pharmacopoeia Commission, Volume II,III Ghaziabad, 6th Edn. Govt. of Indian Ministry of Health and Family Welfare;2010, p. 842,843, 2071, 2072.
3. Maryadele J, Neil O. The Merck Index, Encyclopedia of Chemicals, Drugs and Biologicals, 14th Edn. Merck Research laboratories;2006, p. 8270, 3978.
4. Prajapati P, Bodiwala K, Marolia B, Rathod I, Shah S. Development and Validation of Extractive spectrophotometric method for determination of Rosuvastatin calcium in pharmaceutical dosage forms**.** Journal of Pharmacy Research 2010; 3(8):2036-2038.
5. Kumar MK, Teja GS, Kumar YM. A new validated RP-HPLC method for determination of Rosuvastatin calcium in bulk and pharmaceutical dosage form. Scholars Research Library Der Pharmacia Lettre 2011;3(3):350-356.
6. Devi SU, Latha EP, Nagendra CV. Development and validation of HPTLC method for estimation of rosuvastatin calcium in bulk and pharmaceutical dosage forms. International Journal of Pharma and Bio Sciences 2011;2(2):134-140.
7. Kumar S, Jamadar D, Bhat K, Musmade P, Vasantharaju S. Analytical method development and validation for aspirin. International Journal of Chem.Tech. Research 2010;2(1):389-399.
8. Patel G, Vekariya N. RP-HPLC estimation of Aspirin and Atorvastatin calcium in combined dosage forms. Journal of pharmacy research 2009;2(8):1274-1275
9. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use, Validation of Analytical Procedures: Text and Methodology ICH Q2 (R1). 2005.
10. Rajavel R, Ganesh M, Jagadeeswaran M. RP-HPLC Method for the Simultaneous Determination of Aspirin, Atorvastatin and Pioglitazone in Capsule Dosage Form. Asian J. Research Chem 2008;1(1):40-43
11. Mishra P, Dolly A. Simultaneous Determination of Clopidogrel and Aspirin in phamaceutical dosage forms. Indian Journal of Pharmaceutical Sciences 2006;68:365-368.

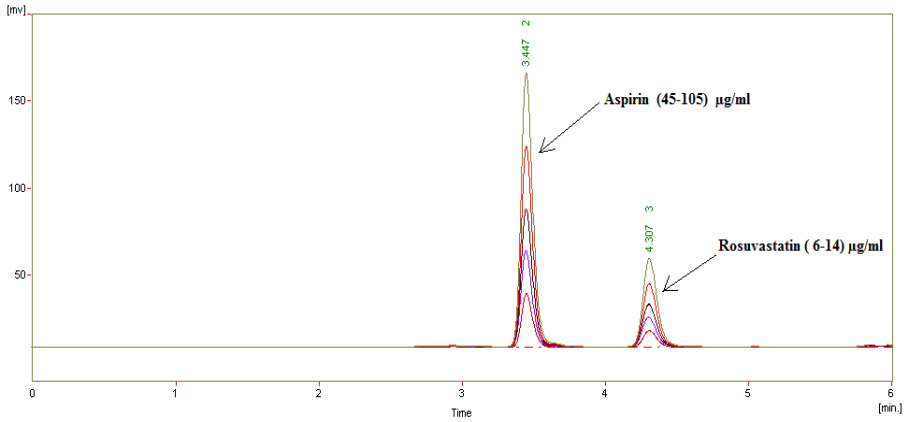

Figure 2: Chromatogram of Aspirin and Rosuvastatin Calcium Mixture

Table 1: Regression Analysis of the Calibration Curves for the Proposed Method

Parameters	RSV	ASP
Linearity Range (µg/ml)	6-14	45-105
Slope	27.72	9.625
Standard deviation of Slope	0.149	0.030
Intercept	-62.89	-162.5
Standard deviation of Intercept	1.60	1.78
Correlation co-efficient	0.9997	0.9989

Table 2: Summary of Validation Parameters

Parameters	RSV	ASP
Limit of detection(µg/ml)	0.50	1.06
Limit of quantification(µg/ml)	1.58	3.30
Accuracy (%)	98.02-100.68	98.38-101.42
Precision(% C.V) Intraday(n=3*3) Interday (n=3*3)	 0.78 - 1.53 0.80 - 1.80	 0.11 - 1.02 0.75 - 1.86
Robustness	1.43	1.56
Repeatability(RSD %)(n=6)	0.87	0.90

Table 3: System Suitability Test Parameters for RSV and ASP by the Proposed Method

System suitability Parameters	RSV	ASP
Retention time (min)	4.30	3.44
Resolution	4.90	
Theoretical plate	>8900	>6000
Tailing Factor (asymmetric factor)	1.25	1.37

Table 4: Assay Results of Combined Dosage Form Using Proposed Method

Formulation	Labelled mg/capsule		Obtained % of label claim ± S.D*	
	RSV	ASP	RSV	ASP
Unistar (unichem)	10	75	101.4 ± 0.86 %	101.56 ± 1.02%

ASP = Aspirin, RSV = Rosuvastatin calcium

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