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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR THE SIMULTANEOUS ESTIMATION OF ROSUVASTATIN AND ASPIRIN DOSAGE IN COMBINE DOSAGE FORM

Shubham D. Chaudhari, Sachin S. Rane, Prof. (Dr.) Rajesh Y. Chaudhari,Prof. (Dr.) Vijay R. Patil
Hon'ble Loksevak Madhukarrao Chaudhari College of Pharmacy, Faizpur. Tal. Yawal Dist. Jalgaon Maharashtra. PIN- 425503.

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ABSTRACT

A simple, robust, precise, UV spectroscopic method has been developed for the simultaneous estimation of Rosuvastatin and Aspirin in bulk and capsule dosage forms. In this paper the estimation of those drugs was carried out by absorbance ratio method. This method is based on measurement of absorption at 239nm and 233nm i.e, λ_{max} of Rosuvastatin and Aspirin respectively. The linearity observed for Rosuvastatin is in the range of 4 to14 µg/ml and for Aspirin is in the range of 20 to 60 µg/ml. The accuracy of method was found to be within the range of 99.62%-99.73% for both Rosuvastatin and Aspirin respectively. The developed method was validated with respect to linearity, accuracy and precision. The method can be employed for estimation of pharmaceutical formulations with no interference from any excipients and diluents. The results were validated as per ICH guidelines.

Corresponding author
Shubham D. Chaudhari
Department of Pharmaceutical Chemistry,
Hon'ble Loksevak Madhukarrao Chaudhari College of Pharmacy,
Faizpur. Tal. Yawal Dist. Jalgaon Maharashtra.
PIN- 425503.
07798222797
chaudharyshubham3212@gmail.com

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INTRODUCTION

Rosuvastatin (ROSA) is calcium salt of (E)- 7- [4- (4- fluorophenyl) - 6- isopropyl- 2- [methyl (methylsulfonyl) amino]pyrimidin- 5- yl] (3R,5S)- 3,5- dihydroxyhept- 6- enoic acid. ROSA is a selective and competitive inhibitor of 3- hydroxy- 3- methylglutaryl- coenzymeA (HMG- CoA) reductase, the rate- limiting enzyme that converts 3- hydroxy- 3- methylglutaryl coenzyme A to mevalonate, a precursor of cholesterol. ROSA is a member of the class of statins, used to treat hypercholesterolemia and related conditions and to prevent cardiovascular disease. It increases the number of hepatic LDL (Low Density Lipoprotein) receptors on the cell surface to enhance uptake and catabolism of LDL. Secondly, ROSA inhibits hepatic synthesis of VLDL (Very Low Density Lipoprotein), which reduces the total number of VLDL and LDL particles.[1,2]

Aspirin (ASP) also known as acetylsalicylic acid, is a salicylate drug, often used as an analgesic, antipyretic, 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.[3,4]

From the extensive literature review, few analytical methods were reported for individual analysis such as UV[5,6,7,8,10]HPLC[11,12,13] and HPTLC[14]and by other methods but there are very few analytical methods were reported in combination UV[15,16,17]HPLC[18,19]

There are several challenges for the quality control of ROSA and ASP in several formulations produced in India inspired the authors to develop simple method for quantification of ROSA and ASP in bulk and formulation by absorption ratio method.

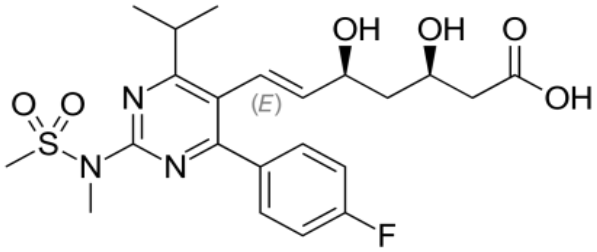

Fig. No. 1. Structure of Rosuvastatin.

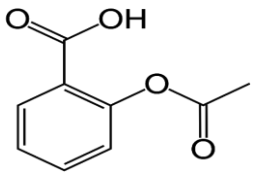

Fig. No. 2. Structure of aspirin.

MATERIALS AND METHODS

Instruments

Shimadzu UV-1800 double beam spectrophotometer was used to record the spectra of sample and reference solutions using pair of Quartz cells of 10mm path length. All weighing was carried out on Shimandzu AUX220 weighing balance. Sonicator of Ultra sonic is used for the purpose of sonication, Filter papers of Sartorius Stedim Biotech of grade 292 are used for the filtration purpose.

Chemicals

The bulk drug of Rosuvastatin was provided by Ajanta pharma, Aurangabad as gift sample and Aspirin procured from Loba Chem. Fixed dose combination capsules (ROSEDAY A 10) capsules containing ROS 10mg and ASP 75mg was procured from local Market All chemicals and reagents of analytical grade and HPLC grade were purchased from USV LTD, Mumbai, India.

Preparation of stock solution and selection of wavelength

Rosuvastatin standard stock solution [R]:

An accurately weighed quantity of ROSA (10 mg) was taken in 10 mL volumetric flask and dissolved in methanol (8 mL) with the help of ultrasonication for about 10 min. Then the volume was made up to the mark using methanol to get Rosuvastain standard stock solution (1 mg / mL).

Rosuvastatin working standard solution [R_1]:

ROSA standard stock solution [R] (1 mL) was diluted to 10 mL using 20% v/v aqueous methanol to get working standard solution (100 µg / mL)

Aspirin standard stock solution [A]:

An accurately weighed quantity of ASP (10 mg) was taken in 10 mL volumetric flask and dissolved in methanol aqueous solution (8 mL) with the help of ultrasonication for about 10 min. Then the volume was made up to the mark using methanol to get Aspirin standard stock solution (1 mg / mL).

Aspirin working standard solution [A_1]:

ASP standard stock solution [A] (1 mL) was diluted to 10 mL using 20% v/v methanol aqueous solution to get working standard solution (100 µg / mL)

Determination of λ max of individual component

An appropriate aliquot portion working standard of ROSA and ASP were transferred to two separate 10 mL volumetric flasks, the volume was made up to the mark using 20% v/v aqueous methanol to obtain ROSA (8 µg/mL) and ASP (60 µg/mL). Drug solutions were scannedseparately between 200 nm to 400 nm ROSA shows the λ_{max} at 239nm while isobestic point shows λ_{max} at 233nm for ROSA and ASP as shown in fig No. 3.

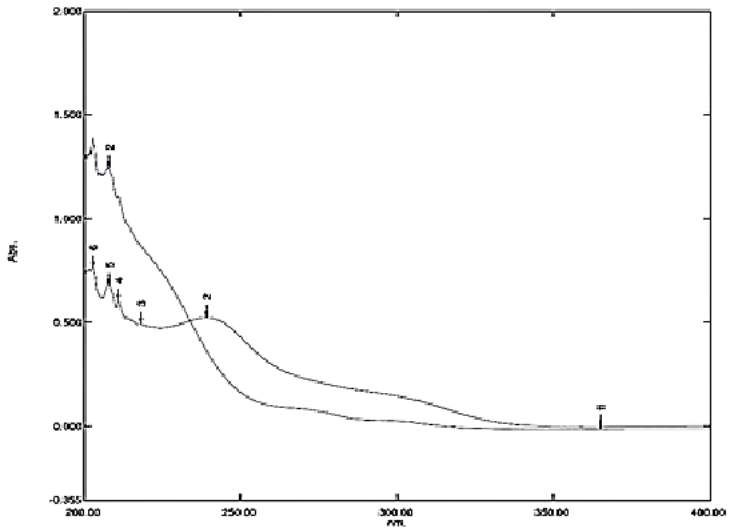

Fig. 3 An Overlay spectra of Rosuvastatin and Aspirin.

Linearity study for Rosuvastatin

An accurately measured aliquot portion of working standard solution of ROSA was transferred to five separate 10 mL volumetric flasks. The volume was made up to the mark using 20% v/v aqueous methanol to obtain concentrations (4µg/ml, 8µg/ml, 10µg/ml, 12µg/ml and 14µg/ml) absorbance measured at 239 nm. Absorbance of these solutions was plotted as absorbance vs concentration. The results are shown in Table No. 1.

Linearity study for Aspirin

Accurately measured aliquot portions of working standard solution of ASP were transferred to seven separate 10 mL volumetric flasks. The volume was made up to the mark using 20% v/v aqueous methanol solution to obtain concentrations (20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml and 60µg/ml) absorbance measured at 233 nm. Calibration curve was plotted, absorbance vs concentration, , the results are shown in the Table No. 1.

Table No. 1: Regression and optical characteristics of ROSA and ASP.

Parameters	Value for Rosuvastatin	Value for Aspirin
Beer's law limit (µg/ml)	4-14 µg/ml	20-60 µg/ml
Regression Coefficient(R^2)	0.9973	0.9997
Regression equation	y= 0.00678x – 0.0062	y= 0.0086 – 0.0034
Slope	0.00678	0.0086
Intercept	0.0062	0.0034

The study of regression and optical characteristics of ROSA and ASP are carried out in which Regression Coefficient (R^2) of ROSA is 0.9973 and of ASP is 0.9997. The slope of ROSA 0.00678 and slope of ASP is 0.0086with Intercept of ROSA 0.0062and for ASP 0.0034, concentration vs absorbance are fairly linear between both co-ordinates by statistical manner and obey ICH guidelines.[20,21]

Estimation of drug by proposed method

The absorbance ratio method[22] is a modification of the simultaneous equations procedure. It depends on the property that, for a substance which obeys Beer's Law at all wavelengths, the ratio of absorbance at any two wavelengths is a constant value independent of concentration or path length.

Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one at isoabsorptive point and other being the λmax of one of the two drugs Rosuvastatin and Aspirin have λmax at 239and 233 nm respectively and isoabsorptive point 233 nm. The wavelengths selected for analysis were 239 and 233 nm, respectively. E (1%,1cm) values of Rosuvastatin and Aspirin were determined at 239 and 233 nm.

The concentration of two drugs in mixture was calculated by using following equations

$$C_{ROSA} = \frac{Qm - Qy}{Qx - Qy} \times \frac{A}{ax1} \quad \text{----------(1)}$$

$$C_{ASP} = \frac{Qm - Qx}{Qy - Qx} \times \frac{A}{ay1} \quad \text{---------- (2)}$$

Where,

$$Qm = \frac{\text{Absorbance of sample at 239 nm}}{\text{Absorbance of sample at 233 nm}}$$

$$Qx = \frac{\text{E (1 \% 1cm) of ROSA at 239 nm}}{\text{E (1 \% 1cm) of ROSA at 233 nm}}$$

$$Qy = \frac{\text{E (1 \% 1cm) of ASP at 239 nm}}{\text{E (1 \% 1cm) of ASP at 233 nm}}$$

Application of proposed method for estimation of drugs in mixture

A quantity of pure drug equal to ROSA (10mg) and ASP (75 mg) was transferred into 100 mL volumetric flask containing methanol (60 mL), sonicated for 15 min and the volume was made up to the mark and filtered through Whatman filter paper (No. 45). This solution was (1 mL) transferred to 10 mL volumetric flaks and volume was adjusted to the mark with 20% aqueous methanol. The absorbance of the solutions was measured at 239nm and 233 nm against blank. The concentration of two drugs was determined by using given equations**.** The results are displayed in the Table No. 2

Table No. 2: Results of estimation of ROSA and ASP in standard laboratory mixture.

Analyte	% Concentration estimated (Mean ± S.D)	% R.S.D
Rosuvastatin	99.79±0.08426	0.08443
Aspirin	99.76±0.073689	0.07385

The estimation of ROSA and ASP in standard laboratory mixture is carried out in which percentage concentration of ROSA and ASP was found to be 99.79 and 99.76 respectively. Those values are fairly accurate by statistical manner and are as per ICH guidelines.

Application of proposed method for estimation of drugs in capsule

Twenty 'ROSEDAY A 10'capsules containing ROSA(10mg) and ASP (75mg) empty capsule shell and contents was weighed and ground to fine powder. A quantity of sample equivalent to ROSA(10mg) and ASP (75 mg) was transferred into 100 mL volumetric flask containing methanol (60 mL), sonicated for 15 min and the volume was made up to the mark and filtered through Whatman filter paper (No. 45). This solution was (1 mL) transferred to 10 mL volumetric flaks, dissolved and volume was adjusted to the mark with 20% aqueous methanol. The absorbance of the solutions was measured at 239nm and 233 nm against blank. The concentration of two drugs in sample was determined by using given equations**.** The results are reported in the Table No.3.

Table No. 3. Results of Estimation of ROSA and ASP in capsule dosage form.

Analyte	Label claim (mg/cap)	% Label claim estimated (Mean±S.D)	% R.S.D
Rosuvastatin	10	99.62± 0.0943	0.0946
Aspirin	75	99.73± 0.1186	0.1189

The results of Estimation of ROSA and ASP in capsule dosage shows form the % purity 99.62and 99.73with SD and RSD bellow 2 which is fairly accurate by statistical manner and are as per ICH guidelines.

Validation of proposed method

The proposed method was validated as per ICH guidelines[20,21].

Accuracy (Recovery study)

Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. A known amount of standard drug solutions were added to the capsule powder to make final concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method. The absorbance recorded and the % recoveries were calculated using formula.

% Recovery = [A - B/ C] X 100

Where,

A = Total amount of drug estimated

B = Amount of drug found on pre analyzed basis

C = Amount of Pure drug added.

The results are reported in the Table No. 4

Table No. 4. Recovery study.

Drug in mixture solution (µg/ml)		% Recovery ± S.D.	
Rosuvastatin	**Aspirin**	**Rosuvastatin**	**Aspirin**
8	20	99.74 ± 1.817	99.72 ± 1.360
10	40	99.78± 1.255	99.84 ± 1.100
12	60	99.69± 1.301	99.87 ± 1.070

The results of Recovery study of ROSA and ASP are found to be fairly accurate between 99.69 to 99.74 % for ROSA and 99.72 to 99.87 % for ASP between various concentrations under observation by statistical way and are obeying ICH guidelines.

Precision

Precision was determined as intra-day and inter-day variations. Intra-day precision was determined by analyzing Rosuvastatin (8, 10 and 12 µg/mL) and Aspirin(20, 40 and 60 µg/mL) for three times on the same day. Inter-day precision was determined by analyzing the same concentration of solutions for three different days over a period of week. The results are shown in the Table No. 5.

Table No. 5. Precision study.

Drug	Conc. [µg/mL]	Intra-day Amount Found		Inter-day Amount Found	
		Mean ±S.D [*n* = 5]	% R.S.D.	Mean ± S.D. [*n* =5]	% R.S.D.
ROSA	8	7.82 ± 0.0870	0.8852	7.85 ± 0.08700	0.8852
	10	9.82 ± 0.2108	1.063	9.82 ± 0.2108	1.063
	12	11.93 ± 0.4624	1.158	11.93 ± 0.4624	1.158
ASP	20	19.88 ± 0.1262	0.6352	19.85 ± 0.1192	0.6005
	40	39.84 ± 0.2590	0.6502	39.88 ± 0.3064	0.7684
	60	59.83 ± 0.082	0.840	59.88 ± 0.07395	0.7479

The Precision Study of ROSA and ASP were carried out and Results are found to be fairly accurate by statistical manner as per ICH guidelines.

Ruggedness

Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by two different analyst using same operational and environmental conditions. The results are reported in the Table No.6.

Table No. 6. Ruggedness Study.

	Rosuvastatin 10 µg/ml		Aspirin 75 µg/ml	
	Amount found in µg/ml Mean ± S.D. (n=3)	% R.S.D	Amount found in µg/ml Mean ± S.D. (n=3)	% R.S.D
Analyst I	9.96 ± 0.2066	0.2584	74.84 ± 0.0953	0.9694
Analyst II	9.75 ± 0.4686	0.5876	74.95 ± 0.1670	1.6787
Day I	9.97 ± 0.2254	0.2819	75.01 ± 0.1081	1.080
Day II	9.81 ± 0.5412	0.6780	74.90 ± 0.1212	1.2124
Instrument I	9.85 ± 0.1184	0.1483	74.996 ± 0.1258	1.2587
Instrument II	9.86 ± 0.1228	0.1538	75.02 ± 0.09643	0.9624

n= 3

The Ruggedness study of ROSA and ASP are carried out and a result are found to be fairly accurate by statistical manner and obeys ICH guidelines.

Lower limit of detection and Lower limit of quantitation

The limit of detection for ROSA and ASP was found to be 0.7652 µg /ml and 1.0435µg /ml respectively and the limit of quantitation of was found to be Rosuvastatin and Asprin2.3189µg /ml and 3.1622µg /ml respectively for given method.

RESULTS AND DISCUSSION

An Absorbance Ratio Method in UV Spectroscopy was developed for ROSA and ASP, the method employs 239nm as λ1 and 233 nm as λ2 for formation of equations. Rosuvastatin and Aspirin obeys Beer's law in the concentration range 4-14 µg/ml (R^2=0.9973) and 20-60 µg/ml (R^2=0.9997) respectively at given wavelengths. The assay estimation in given formulation for ROSA and ASP was found to be 99.62 % and 99.73 % respectively. The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values.

CONCLUSION

The proposed UV spectroscopic method in this paper has advantages of simplicity, accuracy, precision and convenience for quantization of Rosuvastatin and Aspirin. The method can be used for the daily quality control of Rosuvastatin and Aspirin in laboratories and authors recommend further future research on the method before employment of the method.

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CONFLICT OF INTEREST: Hereby authors declare that there is no conflict of interest for this publication.

ABBREIVIATIONS

Selected drug

ROSA : Rosuvastatin
ASP : Aspirin

Symbols

λ max	Wavelength of maximum absorbance
R^2	Correlation coefficient
mL	Milliliter
µg	Microgram
mg	Milligram
g	Gram
nm	Nanometer
%	percentage
>	Greater than
<	Less than
Sec	Second
Min	Minute
Fig	Figure
Temp	Temperature

Other

HPLC	High Performance Liquid Chromatography
UV	Ultra Violet
IP	Indian Pharmacopoeia
ICH	International conference on Harmonization
RSD	Relative Standard Deviation
SD	Standard Deviation
LOD	Limit of Detection
LOQ	Limit of Quantitation

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