

Available online on 15.12.2021 at <http://ajprd.com>

# Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research

© 2013-20, publisher and licensee AJPRD, This is an Open Access article which permits unrestricted non-commercial use, provided the original work is properly cited

Open  Access

Research Article

## Development and Validation of Difference Spectrophotometric Method for Simultaneous Estimation of Rosuvastatin Calcium and Aspirin in Marketed Formulation

**R Padmavathi, Y Padmavathi\*, N Raghavendra Babu, V Akhila, G Keerthi**

Department of Pharmaceutical Analysis, G.Pulla Reddy College of Pharmacy, Osmania University, Hyderabad, Telangana.

### ABSTRACT

**Objective:** To develop and validate a simple Vierordt method for the simultaneous estimation of rosuvastatin calcium (RSV ca) and aspirin (ASP) in fixed dose combination capsules by difference spectrophotometry technique in the UV region. Difference spectrophotometry is based on the utilization of difference absorption spectra corresponding to the same compound obtained at two different pH values.

**Methodology:** The difference in spectral characteristics for the two drugs was observed in 0.01N acetic acid and 0.01N NaOH. The simultaneous equations were developed using difference absorbance and absorptivity values of the two drugs measured and calculated at 243.2nm and 297nm for RSV ca and ASP respectively.

**Results:** Two drugs have shown very good linearity in the concentration range of 10-100µg/ml. The methods were validated as per ICH guidelines for the limit of detection, the limit of quantification, accuracy, and precision, which were found to be within the acceptable limits. The validated method was successfully applied for the analysis of rosuvastatin calcium and aspirin in fixed dose combination capsules.

**Conclusion:** Because this approach is simple and does not require a particular programme, it can be used as a substitute for LC methods in quality control laboratories that lack the necessary equipment for those techniques.

**Keywords:** UV-difference spectrophotometric method, Rosuvastatin calcium, Aspirin, Vierordt's method, Method validation, ICH guidelines.

**ARTICLE INFO:** Received; 05 Sept. 2021; Review Complete; 25 Nov. 2021 Accepted; 29 Nov. 2021 Available online 15 Dec. 2021



#### Cite this article as:

Padmavathi R, Padmavathi Y, Raghavendra Babu N, Akhila V, Keerthi G, Development and Validation of Difference Spectrophotometric Method for Simultaneous Estimation of Rosuvastatin Calcium and Aspirin in Marketed Formulation, Asian Journal of Pharmaceutical Research and Development. 2021; 9(6):56-61

DOI: <http://dx.doi.org/10.22270/ajprd.v9i61046>

#### \*Address for Correspondence:

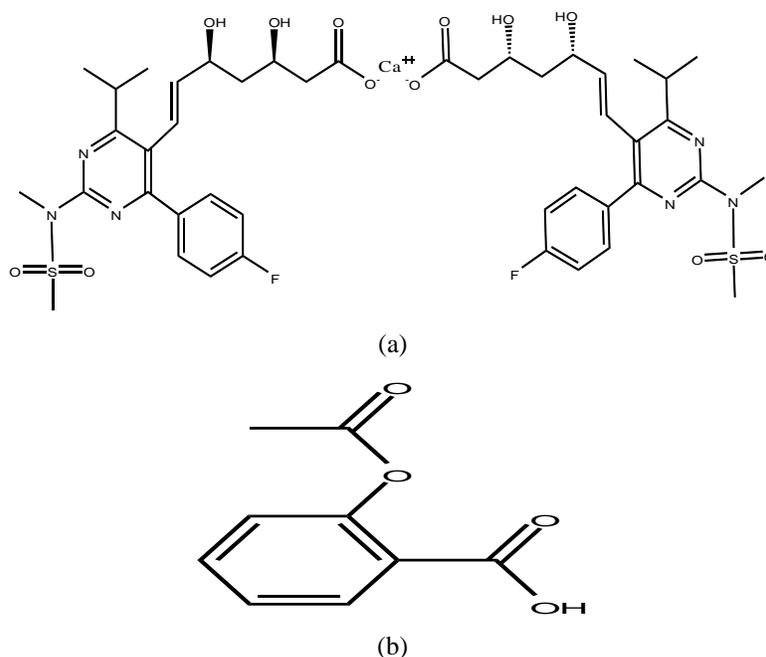
Y Padmavathi, Department of Pharmaceutical Analysis, G.Pulla Reddy College of Pharmacy, Osmania University, Hyderabad, Telangana.

### INTRODUCTION

Hyperlipidemia is a condition in which there are elevated serum levels of one or more of total cholesterol, low-density lipoprotein cholesterol, very low density lipoprotein, triglycerides, or both total cholesterol and total triglycerides (combined Hyperlipidaemia). It is a lifestyle disorder that seriously disturbs human health. It leads to various cardiovascular disorders like angina pectoris, hypertension, atherosclerosis, myocardial infarction, congestive heart failure.<sup>1</sup>

Rosuvastatin calcium (RSV ca) is a statins drug used for the treatment of dyslipidemia. It is also used to lower total

cholesterol, low density lipoprotein-cholesterol (LDL-C), apolipoprotein B (apoB), non-high density lipoprotein (non-HDL-C), and triglyceride (TG) plasma concentrations associated with increased risk of atherosclerosis and cardiovascular disease. It is a semi-synthetic derivative of the naturally occurring compound colchicine with a relaxant effect on skeletal muscle. Aspirin (ASP), also known as acetylsalicylic acid, is an NSAID (Non-steroidal anti-inflammatory drug) used to treat pain, fever, or inflammation which includes pericarditis and rheumatic fever. It is also used long-term to prevent heart attacks, ischaemic strokes, and blood clots.<sup>2</sup> The chemical structure of rosuvastatin calcium and aspirin is shown in *figure 1*



**Figure1:** Chemical structure of (a) Rosuvastatin calcium and (b) Aspirin

Literature review revealed that there are few UV spectrophotometric and HPLC, HPTLC methods reported for simultaneous estimation of rosuvastatin calcium and aspirin in bulk and tablet dosage forms, because of their low solubility (BCS class II drugs), but many methods were reported for the individual drugs.<sup>3-17</sup>

The present work is planned to develop Vierordt's method for the simultaneous estimation of RSVca and ASP in fixed dose combination capsules by difference spectrophotometry technique in UV region because of their solubility issues.

## MATERIALS AND METHODS<sup>18-20</sup>

All the materials and reagents used were of analytical grade. Rosuvastatin calcium and Aspirin were obtained as gift samples from Hetero Labs, Hyderabad. Roseday- A 10 was purchased from the local market. Methanol, Acetic acid, and Sodium Hydroxide are procured from SDFCL, Mumbai.

A Double beam UV-Visible spectrophotometer (SHIMADZU, Japan) 1800 series was used. Data collection and integration were accomplished using UV Probe, 2.43 version software. Other instruments used in the method development and validation, include sonicator (PCI Analytics, 6.5lit 200H), electronic balance (Shimadzu, BL220H).

### Preparation of standard and sample solutions

A mixed stock solution of Rosuvastatin calcium (RSV ca) and aspirin (ASP) was prepared in methanol at a concentration of 2.5 mg/mL.

### Preparation of sample solution

Twenty fixed dose combination capsules (ROSEDAY-A10, containing 10mg rosuvastatin calcium and 75mg aspirin) were weighed and finely powdered. The powder

quantity equivalent to 10mg of RSV ca AND 75mg of ASP was transferred dissolved in methanol and sonicated well for 15minutes. After sonication the volume was made up to the mark with respective solvent to get the concentration of 1000µg/ml RSV ca and 7500µg/ml ASP. This solution was then filtered through Whatman filter paper. The sample stock solution was diluted with 0.01N acetic acid and 0.01N sodium hydroxide to get the concentration of 10µg/ml RSVca and 75µg/ml ASP.

### Development and optimization of simultaneous equation method

The essential feature of a difference spectrophotometric assay is that the measured value is the difference absorbance ( $\Delta A$ ) or amplitude between an adjacent maximum or minimum between two equimolar solutions of the analyte in different chemical forms exhibiting different spectral characteristics. As pH adjustment is the simplest to alter the spectral properties of the analyte, it is used in this method development and attained by using acetic acid and sodium hydroxide respectively for the acidic and alkaline range of pH.

### Selection of solvent and strength of acid and alkali

Different trials were conducted for optimizing the solvent and strength of acid and alkali. Trial I was carried out using a hydrotropic agent (3M Urea) as solvent. As both drugs have poor solubility, hydrotropic agents were used as solvent systems to increase the solubility. 0.1N hydrochloric acid and 0.1N sodium hydroxide were used as acid and alkali. Trial 2 was performed using the same hydrotropic agent used in the first trial (3M Urea) as solvent by changing the strength of acid and alkali to 0.01N. In trial 3, the same hydrotropic agent (3M Urea) as the solvent and 0.1N acetic acid is used in place of hydrochloric acid. In this next trial, the acetic acid strength was changed and looked for the difference in

absorption characteristics. Trial 5 was carried out using methanol as a solvent and solutions were prepared in 0.1N hydrochloric acid and 0.1N sodium hydroxide. In the next trial with methanol as a solvent, the strength of acid and alkali was changed to 0.01N and looked for changes in absorption intensity. In the 8<sup>th</sup> trial methanol as solvent and 0.01N acetic acid and 0.01N sodium hydroxide were used as acid and alkali. The difference absorption spectrum of rosuvastatin and aspirin for optimized conditions was shown in *figure 2*.

### Selection of wavelength

50µg/ml solutions of RSV ca and ASP were prepared by transferring appropriate aliquots of the stock solution into two different 25 ml volumetric flasks. The volume was made up of 0.01 N HCl and 0.01 N NaOH. The difference absorption spectrum taken by keeping a solution of RSV Ca 0.01 N HCl in the reference cell and equimolar solution of RSV Ca in 0.01 N NaOH in the sample cell compartment showed a maximum value of  $\delta A$  at 243.2 nm. The difference absorption spectrum of solutions of ASP showed maximum values of  $\delta A$  at 297 nm.

### Development of simultaneous equations

Simultaneous equations were developed using the following set of equations:

$$\text{At 243.2 nm} \quad A_1 = ax_1bcx + ay_1bcy \quad (1)$$

$$\text{At 297 nm} \quad A_2 = ax_2bcx + ay_2bcy \quad (2)$$

Where,

Cx and Cy are the concentration of rosuvastatin calcium and Aspirin respectively.

A1 and A2 are absorbances at 243.2 nm and 297 nm respectively;

ax1 and ax2 are absorption coefficients of rosuvastatin calcium at 243.2 nm and 297 nm respectively;

ay1 and ay2 are absorption coefficients of Aspirin at 243.2 nm and 297 nm respectively;

b=1 (for measurement in 1cm cells).

### Validation of the method

The developed method was validated according to the ICH guidelines Q2 (R1): Validation of Analytical Procedures: Text and Methodology for the following validation parameters.<sup>21</sup>

### Linearity and range

For proving linearity, working standard solutions were prepared in the concentration range of 10-100µg/ml, and the difference absorbance was measured at 243.2nm and 297nm for both RSV ca and ASP. These concentrations were chosen based on the proportions of both drugs in commercial formulations. The calibration curves were constructed between concentration and difference absorbance values. Linearity was proved by using the

method of least squares. The calculated regression equations and correlation coefficient values for both drugs were reported in *table 1*. The overlay difference absorbance spectra and calibration curves of both drugs are shown in *figure 3-6*.

### Limit of detection and limit of quantification

The Limit of detection and Limit of quantitation was determined using the standard deviation method. The detection limit (DL) or LOD was calculated from the following equation

$$\text{LOD or DL} = 3.3\sigma / S$$

The quantitation limit (QL) or LOQ was calculated from the following equation

$$\text{LOQ or QL} = 10\sigma / S$$

Where,  $\sigma$  = the standard deviation of the response and S = the slope of the calibration curve

### Precision

The precision of the developed method was determined at two levels repeatability and Intermediate precision. Repeatability is the assessment of an analytical method that was performed by analyzing six replicates of a single concentration of 10µg/ml of RSV ca and ASP. Difference absorbance of samples was recorded at 243.2nm and 297nm and the % Relative Standard Deviation (RSD) was calculated. Intermediate precision determined by checking variation of results within the same day (Intra-day) and between days (Inter-day) was reported by calculating relative standard deviation.

The intra-day precision of the proposed method was determined on samples of both the drugs at various concentration levels (40µg/ml, 60µg/ml, 80µg/ml for both RSV ca and ASP) by analyzing three replicates of each sample as a single assay run at 243.2nm and 297nm. The % RSD was calculated for each concentration. Inter-day precision of the developed method was determined by analyzing three replicates of three different concentration samples (40µg/ml, 60µg/ml, 80µg/ml for both RSV ca and ASP) for three consecutive days at 243.2nm and 297nm. The %RSD was calculated and the results are shown in *table 2*.

### Accuracy

The accuracy of the method was determined for drug substances and drug products. Accuracy for drug substance was determined on samples of pure drug solutions at various concentration levels in the range of 80%-120% (48µg/ml, 60µg/ml, 72µg/ml) by analyzing three replicates of each batch in a single assay.

Accuracy of the method for drug product was determined by recovery studies, carried out by adding a known amount of standard drug (60µg/ml) to 80%, 100%, and 120% of test concentration (60µg/ml) prepared from the sample solution. The % recovery was calculated and reported in *table 3*.

### Analysis of fixed dose combination capsules

From the filtrate of sample stock solution (1000µg/ml RSV ca and 7500µg/ml ASP), 0.1ml was transferred into two 10ml graduated tubes separately and the volume was made up to the mark with 0.01N acetic acid in one tube and with 0.01N NaOH in the other tube to get the final

concentration of 10µg/ml RSV ca and 75µg/ml ASP respectively. The difference in absorbance of the prepared solution was measured at 243.2nm and 297nm. The analysis procedure was repeated 6 times with tablet formulations. The amount of drug present in the formulation was calculated using the following formula.

$$\% \text{ Purity} = \frac{\text{Concentration } (\mu\text{g/ml}) \times \text{Dilution factor} \times \text{Average wt of the capsule}}{\text{Weight of sample powder taken (mg)} \times \text{Label claim}} \times 100$$

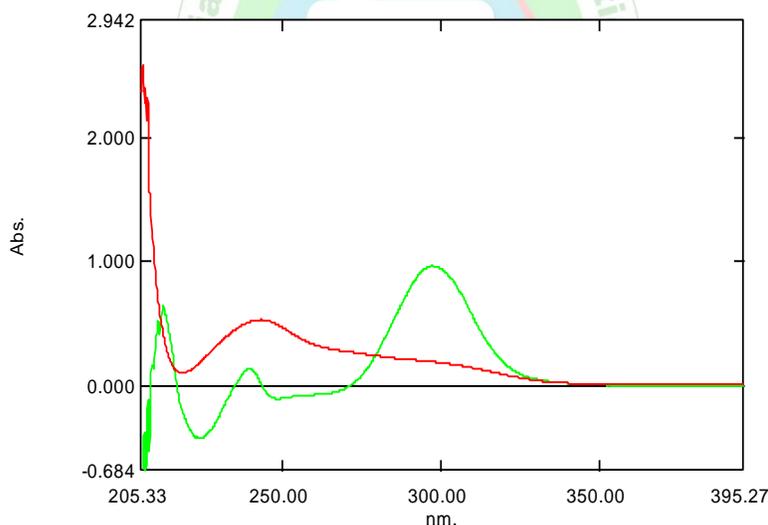
The results of the analysis of formulation were reported in *table 4*.

### RESULTS AND DISCUSSION

Simultaneous equation method was developed and validated for the determination of rosuvastatin calcium and aspirin in fixed dose combination capsules by difference spectrophotometry technique. Changing the pH of the solution is the best option for altering the spectral properties of the analyte, different method development trials were performed for optimizing the type solvent and strength of acid and alkali for bringing pH-induced spectral changes which result in a difference in absorbance. In the above trials, better difference absorbance was observed with 0.01N Acetic acid and 0.01N Sodium hydroxide with methanol as a solvent for stock preparation. The difference

overlay spectra of both the drugs satisfy the criteria for the simultaneous equation method of estimation. So the method was developed and optimized with these conditions. From the selected trial, a bathochromic shift was observed for both rosuvastatin calcium ( $\lambda_{\text{max}}$ -243nm to 243.2nm) and aspirin ( $\lambda_{\text{max}}$ - 257nm to 297nm) in difference absorption spectra.

The difference absorption spectrum of both drugs was taken by scanning 50µg/ml of RSV ca and ASP in the range of 200-400nm. Peak maximum was observed at 243.2 nm for rosuvastatin calcium and 297 nm for aspirin in the overlain difference absorption spectra of the two drugs.



**Figure 2:** Overlay of difference absorption spectra of RSV ca and ASP

Simultaneous equations were developed for the quantification of two drugs by taking difference absorbance and absorptivity values of both drugs at 243.2nm and 297 nm.

Rosuvastatin calcium and Aspirin showed linearity with a absorbance in the range of 10-100 µg/ml at their respective absorption maximum, which was validated by the least

square method. The coefficient of correlation was found to be 0.997 for RSVca and ASP respectively. The optical characteristics and the statistical analysis of the experimental data, the regression equation from the calibration graphs along with standard error of the slopes intercepts and regression coefficient for the difference spectroscopic method is shown in table. 1

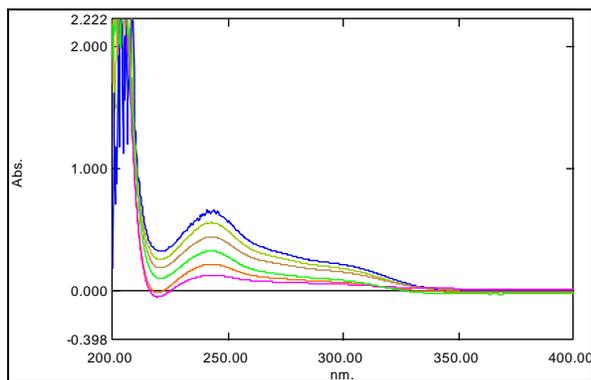


Figure 3: Overlay of the difference absorption spectrum of rosuvastatin calcium

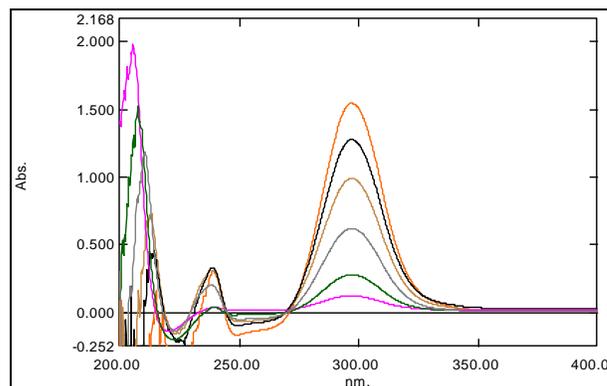


Figure 5: Overlay of the difference absorption spectrum of aspirin

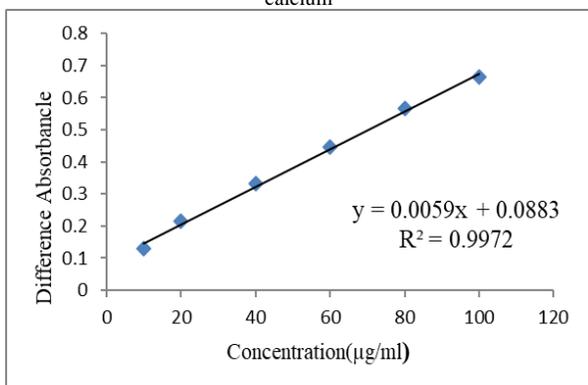


Figure 4: Calibration curve of rosuvastatin calcium

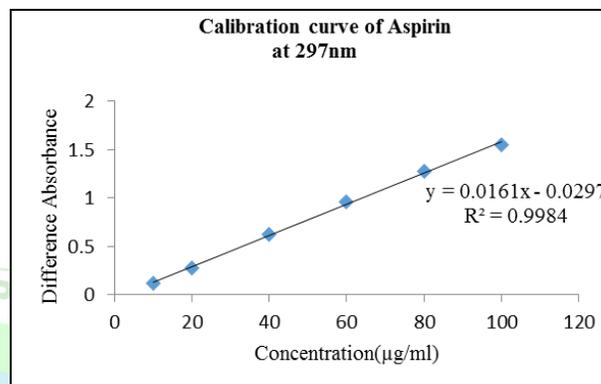


Figure 6: Calibration curve of aspirin at 297nm

Table 1: Optical characteristics of Rosuvastatin calcium and Aspirin

Optical Characteristics	Aspirin	Rosuvastatin calcium
$\lambda_{max}$ (nm)	297	243.2
Beer lambert's law limits (ug/ml)	10-100	10-100
Sandell's sensitivity ( $\mu\text{g.cm}^2/0.001$ abs unit)	0.084	0.079
Limit of detection( $\mu\text{g/ml}$ )	5.21	7.9
Limit of quantification( $\mu\text{g/ml}$ )	15	24.2
Regression equation	$y=0.016x-0.029$	$Y=0.005x+0.088$
Slope (m)	0.016	0.005
Correlation coefficient (r)	0.998	0.997

LOD and LOQ values of both the drugs indicated the sensitivity of the difference spectrophotometric methods. The precision of the developed analytical method was assessed by checking repeatability, intra-day, and inter-day precision. The calculated RSD values are below 2%. The recovery of rosuvastatin calcium and aspirin from the

sample solution was found to be 96.2% and 95% respectively. The recovery results indicate that the method is accurate and the simultaneous equation method can be used for the quantification of these two drugs simultaneously.

Table 2: Repeatability data of RSVca and ASP

S. No.	Concentration( $\mu\text{g/}$		Abs	
	ASP	RSV ca	ASP	RSV ca
1	10	10	0.117	0.131
2	10	10	0.119	0.130
3	10	10	0.123	0.130
4	10	10	0.119	0.131
5	10	10	0.117	0.129
6	10	10	0.123	0.132
Mean			0.119	0.130
Std.deviation			0.0021	0.0011
% RSD			1.8	0.9

**Table 3:** Accuracy data for drug product

Level	Absorbance*		Concentration (µg/ml)		Concentration recovered(µg/ml)		% Recovery	
	ASP	RSV ca	ASP	RSV ca	ASP	RSV ca	ASP	RSV ca
80%	0.712	0.316	48	48	46.18	45.6	96.2	95
100%	1.043	0.425	60	60	66.91	67.4	111	112
120%	1.211	0.460	72	72	77.41	74.5	107	103

\*Average of 3 determinations

**Assay of fixed dose combination tablets**

The developed and validated method was applied for the simultaneous estimation of RSVca and ASP in capsules.

The concentration of both drugs was calculated using simultaneous equations.

**Table 4:** Assay results of formulation

Formulation	Drug	Label claim (mg)	Amount found (mg)	Assay (%)
ROSEDAY	Aspirin		79.81	106
	Rosuvastatin calcium		10.4	104

**CONCLUSION**

The developed difference spectrophotometric method has passed all the validation parameters as per ICH guidelines and can be successfully applied for the simultaneous estimation of rosuvastatin calcium and aspirin in tablet dosage forms using the simultaneous equation method. This method was based on changes in spectral properties of aspirin and rosuvastatin calcium which were studied by changing the pH of the solvent system. This method compensates spectral interferences of other absorbing components.

**ACKNOWLEDGMENT**

The authors thank Hetero Labs, Hyderabad, for supplying gift samples of Rosuvastatin calcium and Aspirin to carry out the work.

**REFERENCES**

- Tripathi KD. Essentials of medical pharmacology. JP Medical Ltd; 2013 Sep 30.
- Pharmacopoeia I. Indian Pharmacopoeia Commission, Ghaziabad, Published by Government of India, Rosuvastatin calcium, III. 2072-2073. Aspirin, II. 2010:842-3
- Singh RD, Yadav H, Hinge M, Patel A. Development and validation of analytical methods for simultaneous estimation of rosuvastatin, clopidogrel, and aspirin in a pharmaceutical dosage form. J Pharm Sci. 2016.
- Eligros Kujur, Madhu Sahu, Neha Dubey, Ajit Pandey, Chanchaldeep Kaur. Validated analytical method development for rosuvastatin calcium by difference spectroscopy. International journal of pharmacy and pharmaceutical research. May 2018; vol.12: issue 2
- Attimarad M, Alkadham A, Almosawi MH, Venugopala KN. Development of rapid and validated RP-HPLC method for Concurrent quantification of rosuvastatin and aspirin Form solid dosage form. Indian J. Pharm. Educ. Res. 2018 Jan 1; 52:151-8
- Savale S, Mahajan H. UV spectrophotometric method development and validation for quantitative estimation of diclofenac sodium. Asian Journal of Biomaterial Research. 2017; 3(2):40-3
- Kumaraswamy G, Lalitha R, Vijayaprakash K. Validated spectrophotometric method for simultaneous estimation of rosuvastatin calcium and aspirin in tablet dosage forms. International Journal of Pharmaceutical Chemistry and Analysis. 2016; 3(2):90-8.
- Kapugandi AN, Gandhi BM, Raju VB. Development and Validation of Stability Indicating RP HPLC Method for Simultaneous Estimation of Ramipril, Aspirin, and Simvastatin in Bulk and Pharmaceutical Dosage Form. Asian J. Biomed and pharm sci. 2016; 6(53):14-20
- Panchal A, Sanghvi G, Vachhani A, Sheth N, Vaishnav D. Simultaneous determination of Aspirin and Rosuvastatin Calcium in capsules by using RP-HPLC coupled with photodiode array detection. International Letters of Chemistry, Physics, and Astronomy. 2014; 14:218-30.
- Purkar AJ, Balap AR, Sathiyarayanan L, Mahadik KR. Development and validation of HPTLC method for simultaneous determination of Rosuvastatin calcium and aspirin in its pure and pharmaceutical dosage form. Int J Pharm Pharm Sci. 2014; 6(5):704-6.
- Reddy SA, Sekhar KC. Development of a UV spectrophotometric method for the simultaneous estimation of rosuvastatin calcium and aspirin in tablets. Journal of global trends in pharmaceutical sciences. 2012; 3(4): 870-875.
- Ambole SR, Shirote PJ, Kondawar MS. Simultaneous Estimation for Rosuvastatin calcium and Aspirin from Capsule Dosage Forms by First Order. Derivative Spectroscopic Method. International Journal of ChemTech Research. 2012;4(3):966-70.
- Patel MJ, Panchal HJ. Simultaneous estimation of aspirin and rosuvastatin calcium in combined dosage form using derivative spectrophotometric method. Int J Pharm Professional's Res.2012; 3(1):540-5.
- Godavariya VD, Prajapati PB, Bodiwala KB, Shah SA. Development and validation of two spectrophotometric methods for the simultaneous estimation of rosuvastatin calcium and aspirin in their combined dosage form. Asian Journal of Research in Chemistry. 2012; 5(2):275-8.
- Purkar AJ, Balap AR, Jadhav SB, Chaudhari PD. Development and validation of UV spectrophotometric method for simultaneous determination of rosuvastatin calcium and aspirin in its pure and pharmaceutical dosage forms. Int J Pharm Chem Sci. 2012; 1(3):1008-12.
- Bhattacharya I, Bhattacharya SP, Talukdar S, Medya S. Development of difference spectrophotometric method for the estimation of aspirin in formulation using hydrotrophy. Int. J.Res. Pharm. Sci. 2011; 2(1):84-7.
- Uyar B, Celebier M, Altinoz S. Spectrophotometric determination of rosuvastatin calcium in tablets. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2007 Jun 1; 62(6):411-3.
- Skoog DA, Holler FJ. TA Nieman Principles of instrumental analysis 5th ed. Saunders College Publ., Philadelphia. 1998.
- Beckett AH, and Stenlake JB. Practical pharmaceutical chemistry. 4th edition, part 2. CBS publishers and distributors. 1997; 284-286,293-296.
- Sethi PD. Quantitative analysis of drugs in pharmaceutical formulations, 3rd ed. CBS publications, New Delhi; 2008: 573-75.
- Guideline ICH. Validation of analytical procedures: text and methodology. Q2 (R1). 2005 Nov; 1(20):05.