Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2012, 4 (3):947-953
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Development and Validation of First Order Derivative Spectrophotometric Method for Simultaneous Estimation of Rosuvastatin Calcium and Aspirin in Capsule Dosage Form

Chiragsinh Solanki*, Nishitkumar Patel, Viral Patel, Dipali Patel and ReetaVaishy

Department of Quality Assurance, Indubhai Patel College of Pharmacy and Research Centre, Dharmaj, Gujarat, India

---

ABSTRACT

A simple, precise, accurate and reproducible spectrophotometric method has been developed for simultaneous estimation of rosuvastatin calcium and aspirin by employing first order derivative zero crossing method in methanol. The first order derivative absorption at 256.3 nm (zero cross point of aspirin) was used for quantification of rosuvastatin calcium and 243.2 nm (zero cross point of rosuvastatin calcium) for quantification of aspirin. The linearity was established over the concentration range of 4-24 µg/ml and 10-60 µg/ml for rosuvastatin calcium and aspirin with correlation coefficient r^2 0.9988 and 0.9983, respectively. The mean % recoveries were found to be in the range of 99.44% – 100.83 % and 99.51– 101.11 % for rosvastatin calcium and aspirin, respectively. The proposed method has been validated as per ICH guideline and successfully applied to the estimation of rosuvastatin calcium and aspirin in their combined capsule dosage form.

Keywords: Rosuvastatin calcium, Aspirin, First order derivative, Method validation

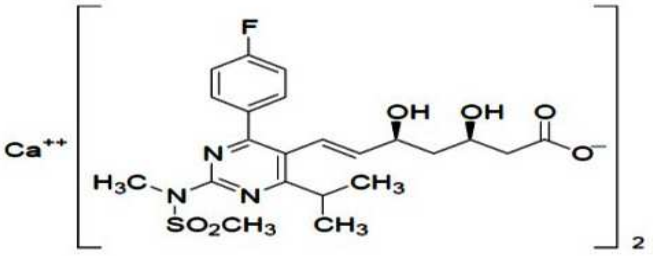
---

INTRODUCTION

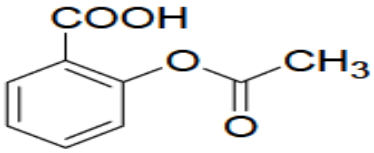
Rosuvastatin calcium (ROS) is chemically (E)-(3R,5S)-7-{4-(4-fluorophenyl)-6-isopropyl-2-{methyl (methyl-sulphonyl) amino]pyrimidin-5-yl}-3,5-dihydroxyhept-6-enoic acid calcium (Figure 1a). ROS is in a group of drugs called hydroxymethylglutaryl coenzyme A (HMG CoA)reductase inhibitors, or "statins." It reduces levels of low-density lipoprotein, apolipoprotein B and triglycerides in the blood, while increasing levels of high-density lipoproteinin the management of hyperlipidaemias[1].Aspirin (ASP) is chemically 2-(acetyloxy)-benzoic acid (Figure 1b). It is non-selective cyclo-oxygenase inhibitor used as an antipyretic, analgesic, anti-inflammatory and antithrombotic agent. It reduces non-fatal myocardial infraction[1-3].ROS and ASP in combined dosage form are used for the treatment of dyslipidemiaassociated with artherssclerotic arterial disease with risk of Myocardial infraction, stroke or peripheral vascular disease.

The review of literature revealed that various analytical methods involving spectrophotometry[4,5], HPLC[6,7], HPTLC[7-9], LC-MS-MS[10], LC-electrospray tandem mass spectrometry[11]have been reported for ROS in single form and in combination with other drugs.Several analytical methods have been reported for ASP in single form and in combination with other drugs including spectrophotometry[12-14], HPLC[15-17], HPTLC[18,19].
The present work describes the development of a simple, precise, accurate and reproducible spectrophotometric

method for the simultaneous estimation of ROS and ASP in capsule dosage forms. The developed method was validated in accordance with ICH Guideline[20] and successfully employed for the assay of ROS and ASP combine capsule dosage form.



(a)



(b)

Figure 1. Chemical structure of ROS (a) and ASP (b)

MATERIALS AND METHODS

Reagents and chemicals

Analytically pure ROS and ASP were kindly provided byRelax Pharmaceuticals, Vadodara, Gujarat, India and Baroque pharmaceuticals, Khambhat, Gujarat, India respectively as gift samples. Analytical grademethanolwas purchased from RFCL limited, New Delhi, India. Capsules of ROS and ASP in combine dosage form, UNISTAR*,with a 10 mg ROS and 75 mg ASP label claim, manufactured by Unichem Laboratories Ltd, India were procured from a local pharmacy.

Instruments

Two spectrophotometers were used for study, A Shimadzu UV/Vis 1800 double beam spectrophotometer with a wavelength accuracy (± 0.3 nm), 1 cm matched quartz cells and UV probe 2.32 software was used for all the spectral measurements and Shimadzu UV/Vis 1601 double beam spectrophotometer with a wavelength accuracy (± 0.3 nm) and 1 cm matched quartz cells was used for reproducibility study.Calibrated analytical balance K-EA 210 (K-Roy Instrument Pvt. Ltd) was used for weighing purpose. All statistical calculations were carried out using Microsoft excel 2010 analytical tool.

Preparation standard stock solutions

Accurately weighed 100 mg of ROS and ASP standard were transferred to separate 100 ml volumetric flask and dissolved in 50 ml methanol. The flasks were shaken and volume was made up to the mark with methanol to give solutions containing 1000 µg/ml ROS and 1000 µg/ml ASP.

Selection of Analytical Wavelength

4 - 24 µg/ml solutions of ROS and 10 - 60 µg/ml solutions of ASP were prepared in methanol by appropriate dilution and spectrum was recorded between 200-400 nm and All zero order spectrums (D^0) were converted to first derivative spectrum (D^1) using delta lambda 1.0 and scaling factor 4.0. The overlain first derivative spectrums of ROS and ASP at different concentration were recorded.The zero crossing point (ZCP) of ROS was found to be 243.2 nm and ZCP of ASP was found to be 256.3 nm.

Method validation

The proposed method has been extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The accuracy was expressed in terms of

percent recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. The precision (% relative standard deviation- %RSD) was expressed with respect to the repeatability, intra-day and inter-day variation in the expected drug concentrations. After validation, the developed methods have been applied to pharmaceutical dosage form.

Specificity
Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into a pre weighed quantity of drugs. The D^1 spectrum was recorded by appropriate dilutions and the quantities of drugs were determined.

Linearity
Appropriate volume of aliquot from ROS and ASP standard stock solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with methanol to give a solutions containing 4-24 µg/ml ROS and 10-60 µg/ml ASP. All D^1 Spectrum were recorded using above spectrophotometric condition. D^1 absorbance at 256.3 nm and 243.2 nm were recorded for ROS and ASP, respectively (n=6). Calibration curves were constructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves.

Accuracy
Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the pre-quantified sample preparation at 3 different concentration levels 80, 100 and 120 %, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times and average recoveries were measured.

Precision
The repeatability was evaluated by assaying 6 times of sample solution prepared for assay determination. The intraday and interday precision study of ROS and ASP was carried out by estimating different concentrations of ROS (12, 16, 20 µg/ml) and ASP (20, 30, 40 µg/ml), 3 times on the same day and on 3 different days (first, second, fifth)and the results are reported in terms of %RSD.

Detection limit and Quantitation limit
ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the $3.3\sigma/S$ and $10\sigma/S$ criterions, respectively; where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve.

Robustness
The sample solution was prepared and then analyzed with change in the typical analytical conditions like stability of analytical solution.

Reproducibility
The absorbance readings were measured at different laboratory for sample solution using another spectrophotometer by another analyst and the values obtained were evaluated using t- test to verify their reproducibility.

Determination of ROS and ASP from combined capsule dosage form
Content of 20 capsules were weighed accurately. A powder quantity equivalent to 75 mg ASP and 10 mg ROS was accurately weighed and transferred to volumetric flask of 100 ml capacity. 50 ml of methanol was transferred to this volumetric flask and sonicated for 15 min. The flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through whatman filter paper (0.45µ). From this solution 1.5 ml was transfer to 25 ml volumetric flask. The volume was adjusted to the mark with the methanol to give a solution containing 6 µg/ml of ASP and 45 µg/ml of ROS. The resulting solution was analyzed by proposed method. The quantitation was carried out by keeping these values to the straight line equation of calibration curve.

RESULTS AND DISCUSSION

First order derivative spectrophotometric method was developed for determination of ROS and ASP. The proposed method has been extensively validated as per ICH guidelines. Summary of validation parameters for proposed method was given in Table 1.

The overlain D^1 spectrum of ROS and ASP at different concentrations revealed that at 256.3 nm (ZCP of ASP) ROS possesses significant D^1absorbance and at 243.2 nm ASP possesses significant D^1absorbance. Considering above facts, wavelength 256.3 nm and 243.2 nm were selected for the estimation of ROS and ASP, respectively (Figure 1-3)

Linearity was assessed for ROS and ASP by plotting calibration curves of the D^1 absorbance versus the concentration over the concentration range 4-24 µg/ml and 10-60 µg/ml, respectively. The correlation coefficients (r^2) for ROS and ASP were found to be 0.9988 and 0.9983, respectively (Table 2). The following equations for straight line were obtained for ASP and ROS.

Linear equation for ROS, Y=0.01195 x – 0.00920
Linear equation for ASP, Y= 0.00740 x - 0.01017

The % recoveries were found to be in the range of 99.58% – 101% for ROS and 99.83% –100.11% for ASP (Table 3).The precision of method was determined by repeatability, intraday and interday precision and was expressed as the %RSD (Table 1), which indicate good method precision.

The Limit of detection for ROS and ASP was found to be 0.87602 µg/ml and 2.62955 µg/ml respectively.Limit of quantification for ROS and ASP was found to be 2.65461 µg/ml and 7.96834 µg/ml at 256.3 nm and at 243.2 nm respectively (Table 1).

The method was also found to be specific, as there was no interference observed when the drugs were estimated in presence of excipients and robust, as there was no significant change in absorbance up to 24 hours of preparation of solution in methanol. The proposed spectrophotometric method was successfully applied to ROS and ASP combined capsule dosage form. The results are shown in Table 6.

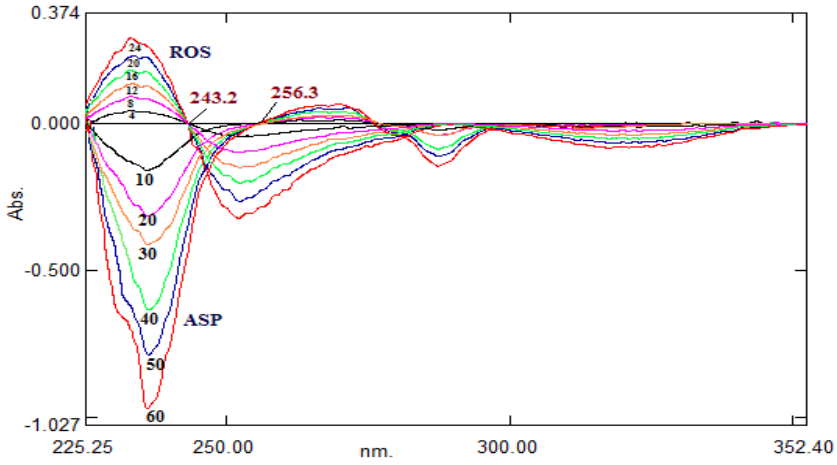


Figure 2.Overlain D^1 spectrum of ROS (4-24 µg/ml) and ASP (10-60 µg/ml) in methanol

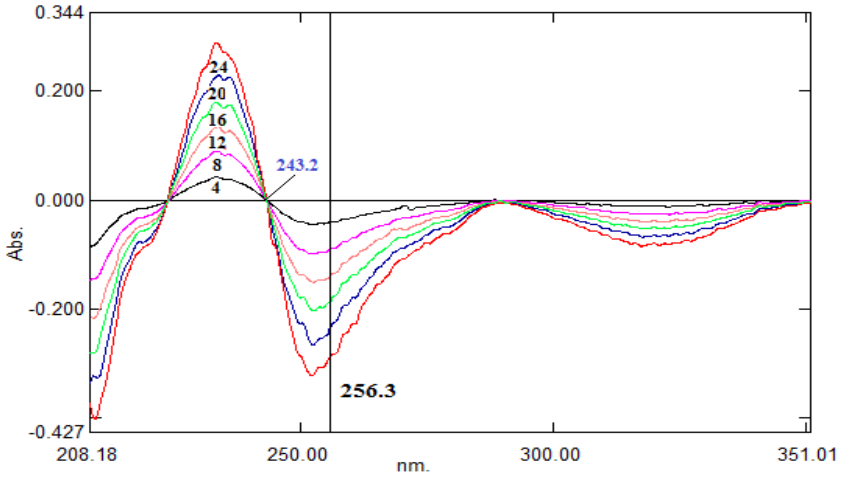


Figure 3.Overlain D[1]spectrum of ROS (4-24 µg/ml) in methanol

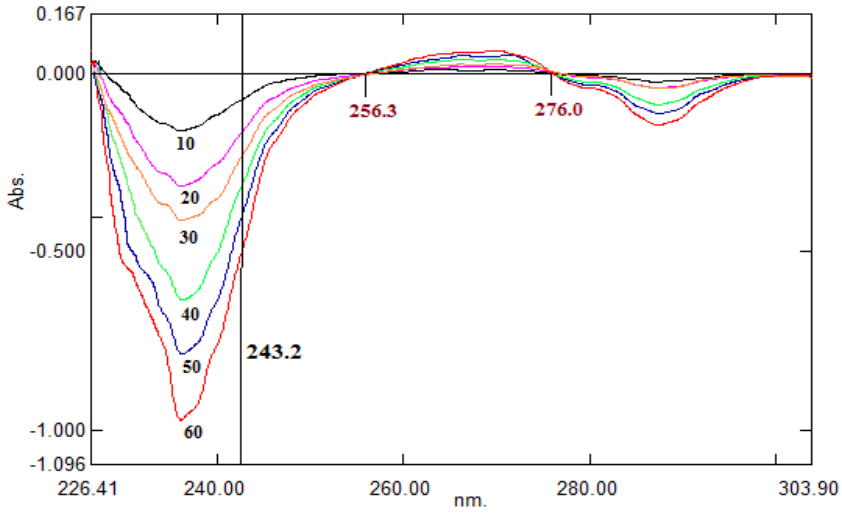


Figure 4 Overlain D[1]spectrum of ASP (10-60 µg/ml) in methanol

Table 1.Summary of Validation Parameters of derivativespectrophotometric method

Parameters	ROS	ASP
Recovery %	99.44 % – 100.83 %	99.51 %– 101.11 %
Precision(% RSD)		
Repeatability (n=6)	0.86256	0.82687
Intra-day (n=3) Inter-day (n=3)	0.43301 – 0.67391 0.74627 – 0.91569	0.47696 – 0.69444 0.89453 – 1.06572
Limit of Detection (µg/ml)	0.87602	2.62955
Limit of Quantitation (µg/ml)	2.65461	7.96834
Specificity	Specific	Specific
Robustness	Robust	Robust
Solvent suitability	Suitable for 24 hrs.	Suitable for 24 hrs.

Table 2. Statistical data for ROS and ASP by derivativespectrophotometric method

Parameter	ROS at 256.3 nm	ASP at 243.2 nm
Linear Range (µg/ml)	4-24	10-60
Slope	0.01195	0.00740
Intercept	-0.00920	-0.01017
Standard deviation of slope	0.00020	0.00015
Standard deviation of intercept	0.0317	0.00590

Table 3. Accuracy data for ROS and ASP by derivative spectrophotometric method

% Level	Amount of drug added (µg/ml)		Amount recovered (µg/ml)		% Recovery	
	ROS (µg/ml)	ASP (µg/ml)	ROS (µg/ml)	ASP (µg/ml)	% ROS	% ASP
80 %	4.8	36	4.84	36.40	100.83	101.11
100 %	6	45	6.01	44.78	100.17	99.51
120 %	7.2	54	7.16	53.91	99.44	99.83

Table 4. Reproducibility data at 256.3 nm for ROS (6 µg/ml)

Instrument 1 Mean ± S.D. (n=3)	Instrument 2 Mean ± S.D. (n=3)	Result of t test[a]	Inference
0.0633 ± 0.0006	0.0637 ± 0.0010	0.67	Not significant difference

[a] At 95% confidence interval, (t-Tabulated = 4.30)

Table 5. Reproducibility data at 243.2 nm for ASP (45 µg/ml)

Instrument 1 Mean ± S.D. (n=3)	Instrument 2 Mean ± S.D. (n=3)	Result of t test[a]	Inference
0.3210 ± 0.0020	0.3227 ± 0.0015	0.53	Not significant difference

[a] At 95% confidence interval, (t-Tabulated = 4.30)

Table 6. Assay Results of Marketed Formulation

Formulation	Actual concentration (µg/ml)		Amount obtained[a](µg/ml)		% ROS	% ASP
	ROS	ASP	ROS	ASP		
Capsule	06	45	06.08	44.84	101.39	99.65

[a]average of3 determination

CONCLUSION

The proposed first order derivative method provide simple, specific, precise, accurateand reproducible quantitative analysis for simultaneous determination of ROS and ASP in combined capsule dosage form. The method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The proposed method can be used for routine analysis and quality control assay of ROS and ASP in combined dosage form.

Acknowledgements
Authors are thankful to Relax Pharmaceuticals (Vadodara, India) for providing gratis sample with the great pleasure and a note of thanks to Baroque Pharmaceuticals (Khambhat, India) for providing gratis sample with the great pleasure. The authors also heartlythankful to Indubhai Patel College of Pharmacy and Research Centre (Dharmaj, India) for funding entire project and providing the necessary facilities for research work.

REFERENCES

[1] Martindale,The Complete Drug Reference, 34th ed., London: Pharmaceutical Press.,**2005**, pp. 15-19, 966.
[2] The Merk Index, Merck Research Laboratories, 14th Ed., Division of Merk& Co., Whitehouse station, USA, **2001**, pp. 17.
[3] Antithrombotic Trialists' (ATT) Collaboration. *Lancet*, **2009**, 373, 1849–1860.
[4] AM. Badawy; NM Mostafa; Abd El-Aziz B;Abd El-Aleem; NT Lamie.*J. Adv. Pharm. Res.*,**2011**, 2, *1*, 44-55.
[5] CB Pandya;Channabasavaraj KP and Shridhara HS.*Int. J. ChemTech Res.*, **2010**, 2, 2140-2144.
[6] HO Kaila; MAAmbasana; RSThakkar; HT Saravaia; AK Shah.*Indian J. Pharm. Sci.*,**2010**, 72, *5*, 592-598.
[7] J Varghese; S Kochupappy; R Thengungal. *J. AOAC Int.*, **2010**, 93, *4*, 1222-1227.
[8] S. Uma Devi; E. PushpaLatha; CVNagendra Kumar Guptha and Mr. P. Ramalingam.*Int. J. Pharma Bio Sci.*,**2011**, 2, *2*, 134-140.
[9] BGChaudhari; NM Patel; PB Shah. *Indian J. Pharm. Sci.*,**2007**, 69, *1*, 130-132.
[10] CK Hull; AD Penman; CK Smith; PD Martin.*J. Chromatogr. B*,**2002**, 772, 219–228.
[11] RK Trivedi; RR Kallem; RMullangi; NR Srinivas.*J. Pharm. Biomed. Anal.*, **2005**, 39, 661–669.
[12] MD Game;KBGabhane; DM Sakarkar. *Indian J. Pharm. Sci.*,**2010**, 72, *3*, 365-368.
[13] ZKokot and KBurda.*J. Pharm. Biomed. Anal.*,**1998**, 18, 871–875.

[14] G Garg; S Saraf and S Saraf. *Indian J.Pharm. Educ. Res.*,**2008**, 42, *1*, 74-77.
[15] DA Shah; KK Bhatt; RS Mehta; MB Shankar; SLBaldania and TR Gandhi.*Indian J. Pharm. Sci.*,**2007**, 69, *4*, 546-549.
[16] ER Montgomery; S Taylor; J Segretario; EEngler; D Sebastian.*J. Pharm. Biomed. Anal.*,**1996**, 15, 73-82.
[17] E. Deconinck; PY Sacre; SBaudewyns; P Courselle; J De Beer.*J. Pharm. Biomed. Anal.*,**2011**, 56, 200-209.
[18] SV Londhe; SV Mulgund; RS Deshmukh; KS Jain. *ActaChromatogr.*,**2010**, 22, 297-305.
[19] HJ Panchal; BN Suhagia; NJ Patel. *J. Planar Chromatogr.* , **2009**, 22, 165-271.
[20] ICH Harmonized Tripartite Guidelines, Validation of analytical procedures: Text and Methodology, Q2(R1), **2005**, Geneva.