Volume 3, Issue1, January 2012
Available Online at www.ijppronline.com
International Journal Of Pharma Professional's Research
Research Article

ISSN NO:0976-6723

SIMULTANEOUS ESTIMATION OF ASPIRIN AND ROSUVASTATIN CALCIUM IN COMBINED DOSAGE FORM USING DERIVATIVE SPECTROPHOTOMETRIC METHOD

Manish j. Patel*, Dr. Hiral j. Panchal

Department of Quality Assurance, Arihant School of Pharmacy and Bio-research Institute, Adalaj, Dist-Gandhinagar, Gujarat, India.

Abstract

A simple and reproducible spectrophotometric method, requiring no prior separation, has been developed for the estimation of Aspirin and Rosuvastatin Calcium in combined dosage form. First order derivative spectroscopy method was adopted to eliminate spectral interference, using 255.50 nm and 288.50 nm as zero crossing points for Aspirin and Rosuvastatin Calcium respectively. Methanol was used as a solvent. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method.

Keywords: - First order derivative spectroscopy, Aspirin (ASP), Rosuvastatin Calcium (ROS).

Introduction

A simple, accurate, and reproducible UV spectrophotometric method for simultaneous estimation of two component drug mixture of Aspirin and Rosuvastatin Calcium in combined dosage form has been developed. Aspirin is 2- (Acetyloxy) benzoic acid[1] (Figure 1), which is best known as an anti-platelet drug. Rosuvastatin Calcium is (E) - (3R, 5S) -7- {4- (4 fluorophenyl) 6 isopropyl 2 {methyl (methylsulphonylamino)] pyrimidin-5 yl} 3, 5 dihydroxyhepten-6-oic acid calcium[2] (Figure 2) and it is used for hypercholesterolaemia[2, 3]. ASP is official in pharmacopeias[4-6], and ROS is official in pharmacopeias[4]. A formulation containing 75 mg of ASP and 10 mg of ROS is available in market (UNISTAR*, Unichem Labs Ltd., Mumbai). A survey of literature revealed that few chromatographic[7-18], HPTLC[19-22] and Spectrophotometric[23-27] and methods are reported for determination of ASP and ROS individually or in combination with other drugs.

Correspondence Address:
Manish j. Patel
Department of Quality Assurance, Arihant School of Pharmacy and Bio-research Institute, Adalaj,
Dist-Gandhinagar, Gujarat, India.
E-mail: manishpatel578@yahoo.com
Ph. no.: +91-8866475473

However there is no method reported so far its simultaneous determination of ASP and ROS from combined dosage form. The present work describes a validated[28], simple, precise and accurate spectrophotometric method for simultaneous estimation of ASP and ROS from combined capsule dosage form.

MATERIALS AND METHODS

Materials

Reference Standards of ASP and ROS were obtained as gift samples from the Astrone Pharmaceuticals Ltd., Ahmedabad and Acme Pharmaceuticals Ltd., Ahmedabad, respectively. The drug sample (capsules), UNISTAR* marketed by Unichem Labs Ltd., Mumbai were procured from local market. All other reagents were of analytical grade for Spectrophotometric method.

Preparation of Standard Solution

Accurately weighed ASP (20 mg) and ROS (10 mg) standards were transferred to a 50 ml volumetric flask, dissolved in and diluted to the mark with methanol to obtain standard stock solution for ASP (400 µg/ml) and ROS (200 µg/ml). Aliquot of the solution (25 ml) was transferred to a 50 ml volumetric flask, and diluted to the mark with methanol to obtain working standard solution for ASP (200 µg/ml) and ROS (100 µg/ml).

Selection of Analytical wavelength

Solution of ASP (60 µg/ml) was prepared in methanol

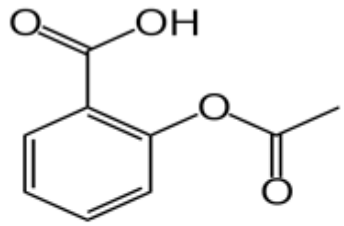

Figure 1. Chemical Structure of Aspirin

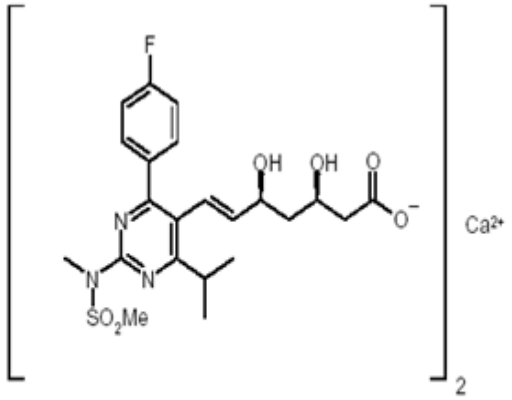

Figure 2. Chemical Structure of Rosuvastatin Calcium

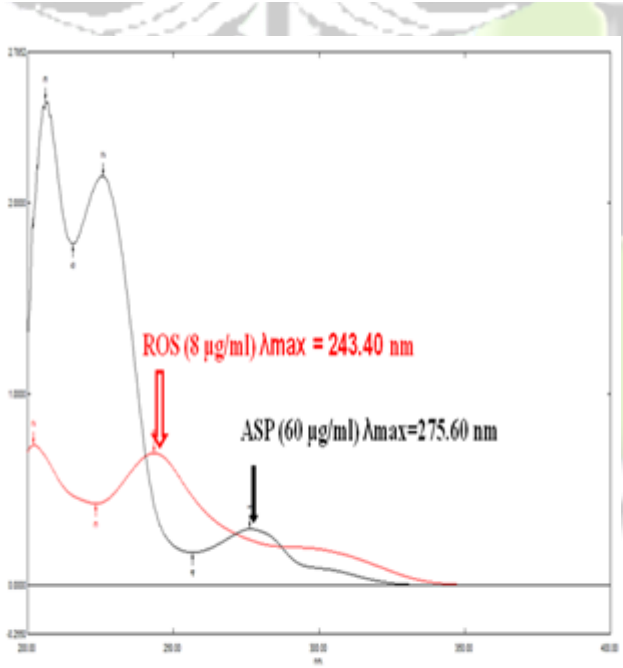

Figure 3. Overlain zero order spectra of ROS (8 µg/ml) and ASP (60 µg/ml) in methanol

and spectrum was recorded between 200-400 nm. First-derivative spectrum for above concentration was obtained. Similarly, Solution of ROS (8 µg/ml) was prepared in methanol and spectrum was recorded between 200-400 nm and first derivative spectrum was obtained. The overlain derivative spectrum of ASP (60 µg/ml) and ROS (8 µg/ml) show the zero crossing point (ZCP) 255..50 nm and 288.50 nm, respectively, which were selected for measurement of ROS and ASP respectively.

Preparation of calibration curve

From the working standard solution appropriate volume of aliquots were transferred to different volumetric flask of 10 ml capacity. The volume was adjusted to the mark with methanol to obtain the concentration of 20, 40, 60, 80, 100, 120 and 140 µg/ml for ASP and 4, 8, 12, 16, 20, 24 and 28 µg/ml for ROS. The samples were scanned between 200-400 nm using SCHIMADZU UV/Visible double beam spectrophotometer (UV-1800) with 1cm matched quartz cells. And spectrums were converted into first order derivative form. Absorbances of ASP and ROS solutions were measured at 288.50 nm and 255.50 nm, respectively using first order derivative spectrophotometric method. The graph of absorbance versus respective concentration was plotted.

Methods

Twenty capsules were accurately weighed and average weight per capsule was calculated. Powder equivalent to 75 mg ASP and 10 mg ROS was accurately weighed and transferred to a 50 ml volumetric flask containing methanol (20 ml). The flask was sonicated for 5 min. The flask was shaken and the volume was diluted to the mark with methanol. The above solution was filtered through Whatman filter paper no. 41. The aliquot 1 ml was transferred to 50 ml volumetric flask and volume adjusted to the mark with methanol. The first derivative response of this solution was measured at 288.50 nm and 255.50 nm for quantification of ASP and ROS, respectively. First order derivative absorbances at these wavelengths were substituted in regression equation representing the calibration curves for ASP and ROS, with correction for dilution, to calculate the amounts of drug present.

RESULTS AND DISCUSSION

Selection of wavelength for simultaneous estimation of ASP and ROS

UV spectra of ASP completely overlaps that of ROS so, absorbance effect of ROS is suppressed in the mixture. Therefore simultaneous estimation in zero order spectra was not successful. So it was thought of interest to develop the first order derivative spectrophotometric method for simultaneous estimation of ROS and ASP from capsule dosage form. Individual first order derivative spectra were recorded for both drugs and zero crossing points were selected (Figure 4). First order derivative spectrum for ROS was taken and it showed zero crossing point 288.50 nm, was selected for determination of ASP in the mixture. Similarly, first order derivative spectrum

for ASP was taken and it showed zero crossing point 255.50 nm, was selected for estimation of ROS in mixture since it showed adequate absorbance at this wavelength.

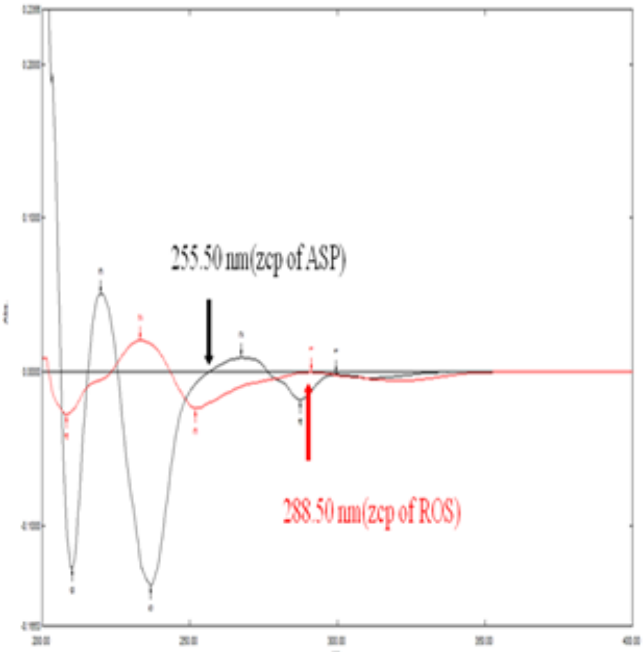

Figure 4. Overlain first order spectra of ROS (8 µg/ml) and ASP (60 µg/ml) in methanol

Validation of the proposed Method

The method is validated as per ICH (International conference on harmonization) Guidelines as follows:

Linearity and Range

The linearity range for both ASP and ROS was found to be in the range of 20-140 µg/ml and 4-28 µg/ml respectively(Figure5).

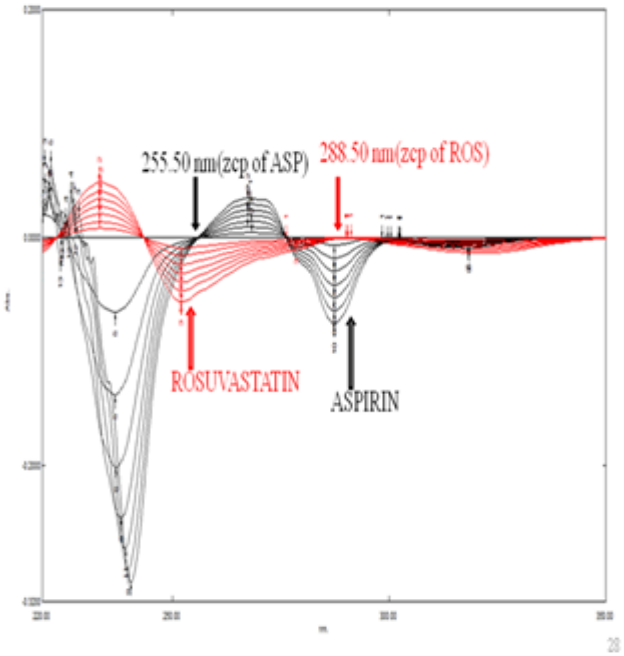

Figure 5. Overlain first order spectra of ROS (4-28 µg/ml) and ASP (20-140 µg/ml) in methanol

Correlation co-efficient for calibration curve of ASP and ROS was found to be 0.9995 and 0.9997 respectively.

The regression line equation for ROS and ASP are as following,

$$Y_{ASP} = 0.0005X - 0.0015 \quad \text{..... (1)}$$

$$Y_{ROS} = 0.0018X - 0.0002 \quad \text{..... (2)}$$

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of ASP and ROS by the standard addition method. Known amount of standards of ASP (15, 30 and 45 µg/ml) and ROS (2, 4 and 6 µg/ml) were spiked to a prequantified sample (30 and 4 µg/ml for ASP and ROS, respectively) and the mixtures were analysed again. The amounts of ASP and ROS were determined by measuring the absorbances and by fitting these values into the regression equation of the calibration plots. The % recovery was found in the range of 98.86 - 100.06 % for ASP (Table 2) and 98.58 - 99.83 % for ROS (Table 3).

Table 1. Linear Regression data of the calibration plots for ASP and ROS (n=3)

Parameter	ASP	ROS
Linearity range (µg/ml)	20-140	4-28
Correlation coefficient (r)	0.9995	0.9997
Slope	0.0005	0.0018
Intercept	0.0015	0.0002

Table 2. Results of recovery studies for ASP (n=3)

Amount of ASP in sample (µg/ml)	Amount of Std ASP added (µg/ml)	Total amount of ASP (µg/ml)	Spicked amount of ASP (µg/ml) (n=3)	% Recovery Mean ± SD	% C.V.
30	15 15 15	45 45 45	14.77 14.94 14.92	98.86 ± 0.63217	0.64
30	30 30 30	60 60 60	29.58 29.66 29.76	98.89 ± 0.30022	0.30
30	45 45 45	75 75 75	45.56 44.85 44.66	100.06 ± 1.05276	1.05

Table 3. Results of recovery studies for ROS (n=3)

Amount of ROS in sample (µg/ml)	Amount of Std ROS added (µg/ml)	Total amount of ROS (µg/ml)	Spicked amount of ROS (µg/ml) (n=3)	% Recovery Mean ± SD	% C.V.
4	2 2 2	6 6 6	1.96 1.97 1.98	98.58 ± 0.61857	0.63
4	4 4 4	8 8 8	3.93 3.99 4.02	99.48 ± 1.11500	1.12
4	6 6 6	10 10 10	5.97 5.99 6.01	99.83 ± 0.33501	0.33

Precision

Repeatability

The repeatability of measurement of absorbance was checked by repeatedly measuring (n = 7) absorbance of same concentration of ASP (60 µg/ml) and ROS (8 µg/ml). The relative standard deviations for the same are 0.72 for ASP; and 1.34 for ROS, respectively (Table 4).

Table 4. Repeatability data for ROS and ASP

Concentration(µg/ml)		% C.V. (n=7)	
ROS	ASP	ASP	ROS
8	60	0.72	1.34

Intermediate precision

The Intermediate precision of the proposed method was assessed by estimating the corresponding responses (n = 3) for 5 different concentrations (20, 40, 60, 80 and 100 µg/ml) for ASP and (4, 8, 12, 16 and 20 µg/ml) for ASP on the same day (Intraday) (Table 5), and on the different days (Interday) (Table 6). The results are reported in terms of relative standard deviation.

Table 5. Intraday precision data for ASP and ROS

Concentration (µg/ml)		% C.V. (n=3)	
ASP	ROS	ASP	ROS
20	4	1.26	1.49
40	8	1.05	1.06
60	12	0.87	0.71
80	16	0.37	0.72
100	20	0.53	0.58

Table 6. Interday Precision data for ASP and ROS

Concentration (µg/ml)		% C.V. (n=3)	
ASP	ROS	ASP	ROS
20	4	1.94	1.71
40	8	1.31	1.40
60	12	1.05	1.18
80	16	0.62	0.75
100	20	0.60	0.76

LOD and LOQ

The limits of detection (LOD) and quantification (LOQ) were calculated from the standard deviation (SD) of y-intercepts and slope (S) of the calibration plots using equations LOD = 3.3 × SD/S and LOQ = 10 × SD/S as per International Conference on Harmonization (ICH) guidelines. The detection and quantification limits obtained by this method were 1.234 and 3.740 µg for ASP; while 0.205 and 0.622 µg for ROS, respectively, which indicates the sensitivity of the method (Table 7).

Table 7. LOD and LOQ

ASP	ROS
LOD=3.3 x (SD/Slope) LOD=3.3x (0.000187/0.0005) LOD=1.234 µg/ml	LOD=3.3 x (SD/Slope) LOD=3.3x (0.0001120/0.0018) LOD=0.205 µg/ml
LOQ=10 x (SD/Slope) LOQ=10 x (0.000187/0.0005) LOQ=3.740 µg/ml	LOQ=10 x (SD/Slope) LOQ=10 x (0.0001120/0.0018) LOQ=0.622 µg/ml

Simultaneous estimation of ASP and ROS in pharmaceutical dosage form

The proposed method was applied to analyze the combined capsule dosage form of ASP and ROS. Marketed preparation was analyzed by the proposed method. The amount of ASP and ROS was found to be 98.88 and 98.61 % of the labeled amount respectively. Thus, the developed first order derivative spectrophotometric method is simple, rapid, precise, accurate and economical. It can be applied for routine analysis of ASP and ROS combined dosage forms.

Table 8. Resuts of Assay of marketed formulation

Brand	Formulation	ASP content	ROS content	Manufacturer	Batch No.	Mfg. date	Expiry date	% label claim (n=3)	
								ASP	ROS
UNISTAR	capsule	75 mg	10 mg	GKM NEW PHARMA	PRA-1003	APR. 2011	MAR.2013	98.88	98.61

CONCLUSION

The proposed first order derivative Spectrophotometric method is accurate, simple, rapid and selective for simultaneous estimation of ASP and ROS in capsule dosage form. Hence it can be conveniently adopted for routine quality analysis of the capsules.

Acknowledgements

We would like thank to Astrone Pharmaceuticals Ltd, Ahmedabad and Acme Pharmaceuticals, Ahmedabad for providing reference sample of Aspirin and Rosuvastatin Calcium respectively to facilitate this work and also to the Principle Dr Ragin Shah, Arihant school of pharmacy and bio-research institute, Adalaj, Dist-Gandhinagar for providing facilities.

REFERENCES

1.The Merck Index, thirteenth Edition. An Encyclopedia of chemicals, Drugs and Biologicals:145, 146.

2.Rang HP, Dale MM, Ritter JM., Flower RJ. Rang and Dale's Pharmacology; 6th Edn; Churchill Livingstone, Elsevier Science Ltd., 2007:323-329.

3.Brunton LL, Parker KL. Goodman and Gilman's Manual of Pharmacology and Therapeutics; 11th Edn; The Mc Graw Hill Companies Inc. 2005:626,709-710.

4.Indian Pharmacopoeia, The Indian Pharmacopoeia Commission, Ghaziabad, Govt. of Indian Ministry of Health and Family Welfare 2010;2,3:636, 2071, 2072.

5.The British Pharmacopoeia, The Department of health, London: The Stationary Office, 2011;II:1468.

6.United States Pharmacopoeia-30, National Formulary-25, Asian Edition, The United States Pharmacopoeia Convention, Rockville, MD, 2007;3101.

7.Reddy GV, Reddy BV, Haque Sw. Development and validation of a stability-indicating UPLC method for rosuvastatin and its related impurities in pharmaceutical dosage forms. Journal Quim. Nova. 2011;34: 250-255.

8.Singh SS, Sharma K, Jain M. Estimation of rosuvastatin in human plasma by HPLC tandem mass spectroscopic method and its application to bioequivalence study. J. of Braz. Chem. Soc. 2005;16:944-950.

9.Zou-Rong R. Quantitative determination of Rosuvastatin in human plasma by liquid chromatography with electrospray ionization tandem mass spectrometry. Rapid Communications in Mass Spectrometry. 2006;20:2369-2375.

10.Lana K, Jiang X, Lia Y. Quantitative determination of rosuvastatin in human plasma by ion pair liquid–liquid extraction using liquid chromatography with electrospray ionization tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis. 2007;44:540-546.

11.Pandya CB, Channabasavaraj KP, Chudasama JD. Development and validation of RP-HPLC method for determination of rosuvastatin calcium in bulk and pharmaceutical dosage form. International Journal of Pharmaceutical Sciences Review and Research. 2010;5:82-86.

12.Kaila HO, Ambasana MA, Thakkar RS. A new improved RP-HPLC method for assay of rosuvastatin calcium in tablets. Indian journal of pharmaceutical sciences. 2010;72:592-598.

13.Sultan N. Simultaneous determination of atenolol, rosuvastatin, spironolactone, glibenclamide and naproxen sodium in pharmaceutical formulations and human plasma by RP-HPLC. Journal of the Chinese Chemical Society. 2008;55:1022-1029.

14.Gajjar AK, Shah VD. Development and Validation of a Stability-Indicating Reversed_Phase HPLC Methood for Simultaneous Estimetion ol rosuvastatin and ezetimibe from their combination dosage forms. Eurasian journal of analytical chemistry. 2010;5(3).

15.Sudhakari M, Rao JV. A New Improved Rp-Hplc Method For Simultaneous Estimation Of Rosuvastatin Calcium And Fenofibrate In Tablets. International Journal of Pharmacy and Pharmaceutical Sciences. 2011;3.

16.Sevda RR, Ravetkar AS, Shirote PJ. UV Spectrophotometric estimation of Rosuvastatin Calcium and Fenofibrate in bulk Drug and Dosage Form using Simultaneous Equation Method. International Journal of ChemTech Research. 2011;3(2):629-635.

17.Pandya CB, Channabasavaraj KP, Shridhara HS. Simultaneous Estimation of Rosuvastatin Calcium and Ezetimibe in Bulk and Tablet Dosage Form by International Journal of ChemTech Research.2010; 2(4):2140-2144.

18.Miti SS, Pavlovi AN, Sunari SM. Quantitative Analysis of Acetylsalicylic Acid in Commercial Pharmaceutical Formulations and Human Control Serum Using Kinetic Spectrophotometry. *Acta. Chim. Slov*. 2008**;**55:508–515.

19.Raj HA, Rajput SJ. Development and validation of two chromatographic stability-indicating methods for determination of rosuvastatin in pure form and pharmaceutical preparation. International Journal of ChemTech Research. 2009;1:677-689.

20.Saner T. Determination of rosuvastatin calcium in its bulk drug and pharmaceutical preparations by high-performance thin-layer chromatography. Journal of planar chromatography. 2005;18:194-198.

21.Sharma MC, Sharma S, Kohlib DV,Sharma AD. A validated HPTLC method for determination of simultaneous estimation Rosuvastatin Calcium and Ezetimibe in pharmaceutical solid dosage form. Archives of Applied Science Research. 2010;2(1):1-7.

22.Panahi HA, Rahimi A, Mohiri E, Izadi A, Parvin MM. HPTLC separation and quantitative analysis of aspirin, salicylic acid, and sulfosalicylic acid. JPC-Journal of Planar Chromatography-Modern TLC. 2010;23:137-140.

23.Gupta A. Simple uv spectrophotometric determination of rosuvastatin calcium calcium in pure form and in pharmaceutical formulations. E-Journal of Chemistry. 2009;6:89-92.

24.Altinoz S. Spectrophotometric determination of rosuvastatin calcium in tablets. J. AOAC Int. 2007;62:411-413.

25.Kumar SS et al. Analytical Method Development And Validation For Aspirin. International Journal of ChemTech Research. 2010;2(1):389-399**.**

26.Patel GF, Vekariya NR, Dholakiya RB. Estimation of Aspirin and Atorvastatin Calcium in Combine Dosage Form Using Derivative Spectrophotometric Method. International Journal of Pharmaceutical Research. 2010;2(1).

27.Gujarathi SC, Shah AR, Jagdale SC, Datar PA, Choudhari VP, Kuchekar BS. Spectrophotometric Simultaneous Determination of aspirin And Ticlopidine In Combined Tablet Dosage Form By first Order Derivative Spectroscopy, Area Under Curve (Auc) And ratio Derivative Spectrophotometric Methods. International Journal of Pharmaceutical Sciences Review and Research. August 2010;3:115-119.

28.ICH Steering Committee. 2005. International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use; Validation of Analytical Procedure-Methodology, Geneva, Switzerland.