An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF VALSARTAN AND ROSUVASTATIN CALCIUM IN SYNTHETIC MIXTURE

Pinky Rajput*, Darshil. B. Shah, Dilip Maheshwari

L.J. Institute of Pharmacy, Nr. Sanand Cross Roads, Sarkhej-Gandhinagar Highway, Ahmedabad Gujarat-382210, INDIA

*Corresponding author E-mail: pinkyrajput865@gmail.com

ARTICLE INFO

Key Words

Valsartan, Rosuvastatin calcium, First order derivative, Synthetic Mixture

ABSTRACT

The present work was aimed at method development and validation for simultaneous estimation of Valsartan and Rosuvastatin calcium by First order derivative Spectrophotometric method in synthetic mixture. In UV method Methanol is used as solvent. The method was validated in terms of linearity, accuracy and precision, limit of detection (LOD) and limit of quantitation (LOQ) as per ICH (Q2 R1) guideline. To overcome the problem of spectral interference First order derivative spectroscopic method were adopted. The selected wavelengths by First order derivative were found to 223 nm and 250 nm for Valsartan and Rosuvastatin calcium. The linearity range of Valsartan and Rosuvastatin calcium were found to be in the range of 8-40 µg/ml and 1-5 µg/ml respectively. The correlation coefficient value for Valsartan 0.9981 and Rosuvastatin calcium 0.9972. The LOD and LOQ value were found to be 1.04 µg/ml, 3.16 µg/ml for Valsartan and 0.102 µg/ml, 0.309 µg/ml for Rosuvastatin calcium.

INTRODUCTION:

Valsartan is an antihypertensive agent known as angiotensin II receptor antagonist (ARB), which is selective for the type I (AT1) angiotensin receptors. Valsartan is used for the treatment of high blood pressure and congestive heart failure. It blocks the blood pressure by increasing effects of AT2 via the Renin-angiotensin- aldosterone system (RAAS).Valsartan is an orally active non-peptide Triazole derived antagonist of angiotensin (AT) II with antihypertensive properties. Valsartan specifically and competitively blocks the binding of AT2 to the AT1 subtype receptor in vascular smooth muscle and the adrenal gland, preventing AT II - mediated vasoconstriction, aldosterone synthesis & secretion, renal reabsorption of sodium, resulting in vasodilation, increased excretion of sodium & water, a reduction in plasma volume, and a reduction in blood pressure. Rosuvastatin calcium is a drug which belongs to class of statin with antilipidemic activity. The use of Rosuvastatin is for treatment of dyslipidemia. It works by three mechanism 1) Inhibiting cholesterol synthesis, 2) Increasing LDL uptake, 3) Decreasing of specific protein prenylation. It attach and inhibits hepatic hydroxy methyl-glutaryl coenzyme A (HMG CoA) reductase, the enzyme which catalyzes the conversion of HMG-CoA to mevalonate,

a precursor of cholesterol. This help to a decrease in hepatic cholesterol levels and increase in uptake of LDL cholesterol. It is useful for the prevention of cardiovascular disease and to high cholesterol and related condition in combination with exercise, diet, and weight loss. Combination of antihypertensive and statin found to be effective in reducing DBP (diastolic blood pressure). Target BP and increases the LDL (Low density lipid) achievement rate. Combination of the Valsartan and Rosuvastatin calcium showed effective result in relief of hypertension and dyslipidemia. From the literature survey, it was observed that various methods are reported for analysis of Valsartan and Rosuvastatin calcium, individually as well as in combination with other drugs. But no analytical method has been reported for analysis of Valsartan and Rosuvastatin calcium in synthetic mixture. A successful attempt has been made to estimate two drugs simultaneously by First order derivative Spectrophotometric method.

MATERIAL AND METHODS: [5-9]

Instruments

UV Visible Spectrophotometer: A Shimadzu UV–visible double beam spectrophotometer model 1800 (Japan) with spectral width 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell. Spectra were automatically obtained by UV probe system software (UV probe version 2.31)

Digital analytical weighing balance: Wenser DAB-220

Sonicator: Equitron

Chemicals and Materials: Rosuvastatin calcium and Gemigliptin as a gift sample were supplied by (West cost Pharma, Ahmedabad, Gujarat) and (Torrent, Ahmedabad, Gujarat). Methanol (Aventor Performance Material, India) (AR Grade)

Selection of a Solvent: Both The Drugs were soluble in Methanol. So, Methanol was selected as a solvent for estimation of both the Drugs.

U.V Spectrophotometric Method: First-order derivative method for Valsartan and rosuvastatin calcium.

Experimental work

Preparation of standard stock solution

Preparation of standard stock solution of Valsartan (100µg/ml): Accurately weighed 10 mg of Valsartan and transferred into 100 ml volumetric flask, diluted with Methanol and sonicated and made up to the mark with Methanol. (1000µg/ml). Preparation of standard stock solution of Rosuvastatin calcium (100µg/ml): Accurately weight 10 mg of Rosuvastatin calcium was transferred into a 100 ml volumetric flask and diluted with Methanol.

Selection of Wavelength: 1.6 ml working standard stock solution of Valsartan and 0.2 ml working standard stock solution of Rosuvastatin calcium (100 µg/ml) was transferred in to different 10 ml volumetric flask and dilute up to mark with Methanol to get 16 µg/ml and 2 µg/ml of Valsartan and Rosuvastatin calcium. Each solution was scanned in the range of 200-400 nm. Zero order spectra were converted in to First Order spectra. Valsartan shows ZCP (Zero Crossing Point) at 250 nm and Rosuvastatin calcium show ZCP at 223 nm. Hence, these wavelength 250 and 223 were selected as analytical wavelengths.

Preparation of Calibration Curve:

- **Calibration Curve for Valsartan (8-40 µg/ml):**

An aliquots of stock solution of Valsartan (100 µg/ml) 0.8, 1.6, 2.4, 3.2, 4.0 ml was pipette out in 5 different 10ml volumetric flask and was made up to the mark with Methanol which will give 8, 16, 24, 32, 40 µg/ml respectively. Absorbance of each solution was measured at 250 nm (ZCP) using first order derivative Spectrophotometry. Graph of Absorbance vs. Concentration was plotted.

- **Calibration Curve for Rosuvastatin calcium:** An aliquots of stock solution of Rosuvastatin calcium (100 µg/ml) 0.1, 0.2, 0.3, 0.4, 0.5 ml was pipette out in 5 different 10ml volumetric flask and was

made up to the mark with Methanol which will give 1,2, 3, 4, 5 µg/ml respectively.

Absorbance of each solution was measured at 223 nm (ZCP) using first order derivative Spectrophotometry. Graph of Absorbance vs. Concentration was plotted.

Method Validation: The developed method was validated with respect to linearity, accuracy, and precision, limit of detection and limit of quantification in accordance with the ICH guideline.

- **Linearity and Range (n=6):** The linearity of valsartan and Rosuvastatin calcium was found to be in the range of 8-40 µg/ml and 1-5 µg/ml, respectively. Linearity of both the drugs was checked in term of slope, intercept and correlation coefficient.

Precision

1. **Intraday precision (n=3):** Solution containing Valsartan 16, 24, 32 µg/ml and Rosuvastatin calcium 2, 3, 4 µg/ml were analyzed three times on the same day and %R.S.D was calculated.
2. **Interday precision (n=3):** Solution containing Valsartan 16, 24, 32 µg/ml and Rosuvastatin calcium 2, 3, 4 µg/ml were analyzed three times on analyzed on three different successive days and %R.S.D was calculated.
3. **Repeatability (n=6):** Solutions containing of 16 µg/ml of Valsartan and 2 µg/ml Rosuvastatin calcium were analyzed three times on the same day and % R.S.D. was calculated. %R.S.D was not more than 2%.

- **Limit of detection (LOD):** The L.O.D. was estimated from the set of calibration curves of Valsartan and Rosuvastatin used to determine method linearity. Limit of detection can be calculated using following equation as per ICH guidelines.

$$\mathbf{LOD = 3.3\ \sigma/S}$$

Where,

σ = Standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

- **Limit of Quantitation:** The L.O.Q. was estimated from the set of calibration curves of Valsartan and Rosuvastatin calcium used to determine method linearity. Limit of quantitation can be calculated using following equation as per ICH guidelines.

$$\mathbf{LOQ = 10\ \sigma/S}$$

Where,

σ = Standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

- **Accuracy (Recovery study) (n=3)**

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as conventional the value or an accepted reference value found. Accuracy of the developed method concentration levels 50%, 100%, 150%.

Assay:

Preparation of Synthetic Mixture of Valsartan and Rosuvastatin calcium:

The synthetic mixture of Valsartan and Rosuvastatin was prepared in ratio of 160:20

Common excipients: Microcrystalline cellulose, Lactose, Magnesium stearate, Talc with drug Valsartan160 mg and Rosuvastatin calcium 20 mg.

Accurately weighed equivalent weight of Valsartan (160 mg) and Rosuvastatin calcium (20 mg) which transferred in 100 ml volumetric flask and make up half mark with Methanol. Then this concentration of Valsartan is 1600 µg/ml and Rosuvastatin calcium 200 µg/ml.

Preparation of Sample Solution: From the above synthetic mixture 0.2 ml was pipetted out in volumetric flask and made up to the mark with Methanol to make final concentration of Valsartan 16 µg/ml and Rosuvastatin calcium 2 µg/ml.

RESULT AND DISCUSSION:

Selection of wavelength for Valsartan and Rosuvastatin calcium: Valsartan (16 µg/ml) and Rosuvastatin calcium (2 µg/ml) solution were scanned between 400-200 nm. Absorbance maximum were obtained at their λmax 250 nm and 223 nm for Valsartan and Rosuvastatin calcium, respectively.

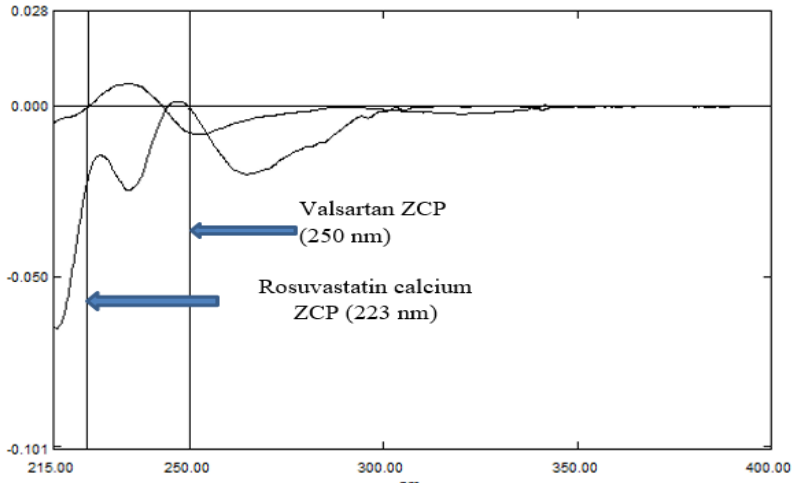


FIGURE 1: first order spectra of Valsartan (16µg/ml) shows ZCP at 250 nm and Rosuvastatin calcium (2µg/ml) shows ZCP at 223 nm in Methanol.

METHOD VALIDATION:

Linearity and range:

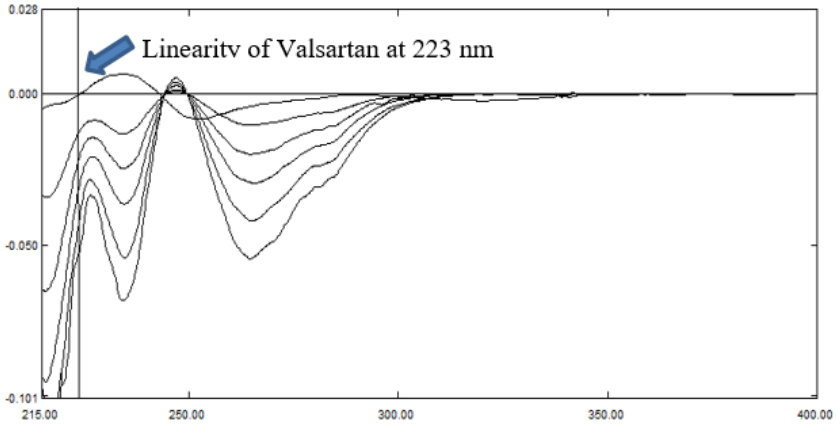


FIGURE 2 Linearity of 1st Derivative spectra of Valsartan (223 nm)

TABLE: 1 Linearity data of Valsartan

Valsartan		
Concentration (µg/ml)	Mean Absorbance ± SD	%RSD
8	|-0.0112|± 0.00010	1.453
16	|-0.0182|± 0.00013	1.252
24	|-0.0273|± 0.00019	1.130
32	|-0.0364|± 0.00021	0.881
40	|-0.0449|±0.00024	0.792

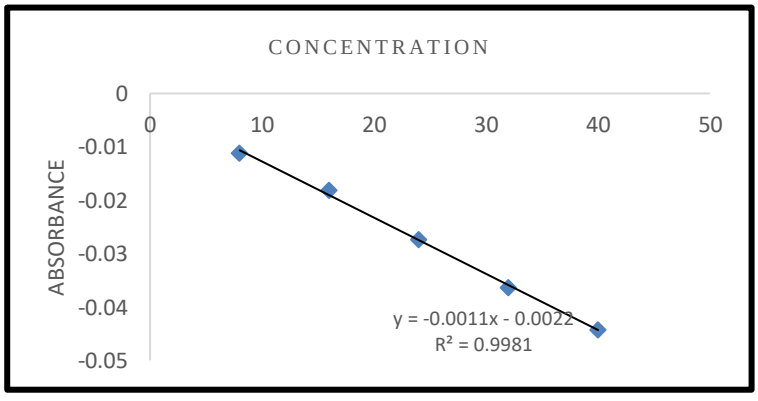


FIGURE 3 Calibration curve of Valsartan at 223nm

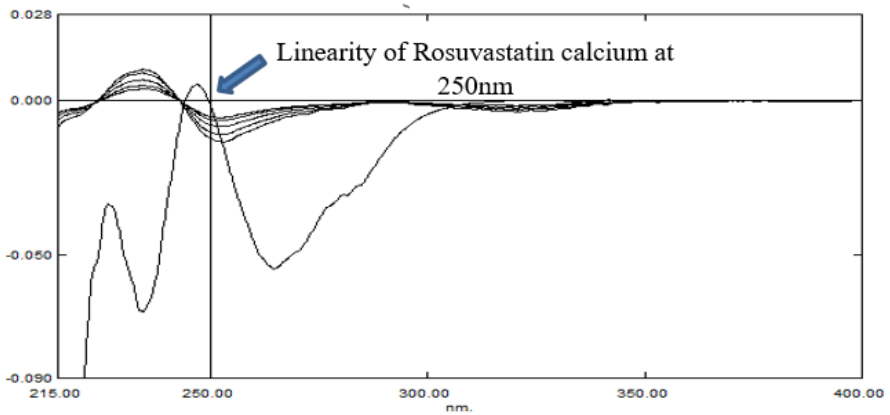
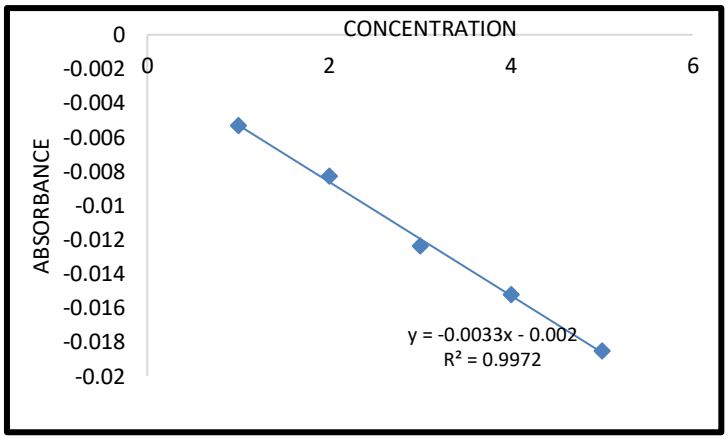


FIGURE 4: Linearity of 1st Derivative spectra of Rosuvastatin calcium (250 nm)



Calibration curve of Rosuvastatin calcium at 250 nm

TABLE: 2 Linearity data of Rosuvastatin calcium

Rosuvastatin calcium		
Concentration (µg/ml)	Mean Absorbance ± SD	%RSD
1	|-0.0053|± 0.00010	1.936
2	|-0.0082|± 0.00013	1.604
3	|-0.0123|± 0.00019	1.567
4	|-0.0152|± 0.00021	1.418
5	|-0.0186|±0.00024	1.341

➢ PRECISION:

TABLE 3: Precision study of Valsartan at 223 nm

Intraday Precision of Valsartan (n=3)		
Conc. (µg/ml)	Mean Absorbance ±SD (n=3)	% RSD
8	|-0.0137|±0.00015	1.109
16	|-0.0222|±0.00023	1.037
24	|-0.0312|±0.00025	0.804
Interday Precision of Valsartan (n=3)		

Conc. (µg/ml)	Mean Absorbance ±SD (n=3)	% RSD
8	|-0.0151|±0.00017	1.372
16	|-0.0253|±0.00025	1.205
24	|-0.0333|±0.00028	1.082
Repeatability of Valsartan (n=6)		
Conc. (µg/ml)	Mean Absorbance ±SD (n=6)	% RSD
16	|-0.0233|±0.00032	1.395

TABLE 4: Precision study of Rosuvastatin calcium at 250 nm

Intraday Precision of Rosuvastatin calcium (n=3)		
Conc. (µg/ml)	Mean Absorbance ±SD (n=3)	% RSD
1	|-0.0064|±0.00011	1.794
2	|-0.0094|±0.00015	1.613
3	|-0.0131|±0.00020	1.584
Interday Precision of Rosuvastatin calcium (n=3)		
Conc. (µg/ml)	Mean Absorbance ±SD (n=3)	% RSD
1	|-0.0076|±0.00014	1.887
2	|-0.00115|±0.00020	1.739
3	|-0.0141±0.00023	1.634
Repeatability of Rosuvastatin calcium (n=6)		
Conc. (µg/ml)	Mean Absorbance ±SD (n=6)	% RSD
2	|-0.0115|±0.00019	1.719

- **LOD and LOQ**

TABLE 5: LOD and LOQ for Valsartan and Rosuvastatin calcium

Parameter	Valsartan	Rosuvastatin calcium
LOD (µg/ml)	1.04	0.102
LOQ (µg/ml)	3.16	0.309

- **Accuracy**

TABLE 6: Recovery study

Name of Drug	% Level of recovery	Test Amount (µg/ml)	Amount of drug taken (µg/ml)	Total Amt (µg/ml)	Total amount Recovered (µg/ml)	%Recovery ± SD (n=3)
Valsartan	50	16	8	24	23.83	98.64±0.568
	100	16	16	32	31.8	98.66±0.612
	150	16	24	40	39.33	98.66±0.603
Rosuvastatin calcium	50	2	1	3	2.98	98.94±0.527
	100	2	2	4	3.93	98.78±0.606
	150	2	3	5	4.96	99.00±0.599

TABLE 7: Analysis of synthetic mixture

Name of drug	Amount taken (µg/ml)	Mean Amount found (µg/ml)	%Assay ± S.D. (n=3)
Valsartan	16	15.83	98.85±0.3867
Rosuvastatin calcium	2	1.96	99.11±0.4122

TABLE 8: Summary of validation parameter

S.no.	Parameters	Valsartan	Rosuvastatin calcium
1	Wavelength (nm)	223 nm	250 nm
2	Beer's Law Limit	8-40 (µg/ml)	1-5 (µg/ml)
3	Regression equation (y = mx +c)	y = -0.0011x - 0.0022	y = -0.0033x -0.0022
4	Correlation Coefficient (r^2)	0.9981	0.9972
5	Intraday Precision (%RSD, n=3)	1.109-0.804	1.794-1.584
6	Interday Precision (% RSD, n=3)	1.372-1.08	1.887-1.634
7	Repeatability (% RSD, n=6)	1.395	1.719
8	Accuracy (% Recovery, n=3)	98.80-98.58	99.28-98.92
9	LOD (µg/ml)	1.04	0.102
10	LOQ (µg/ml)	3.16	0.309
11	%Assay	98.85	99.11

RESULT AND DISCUSSION:

A Simple, Precise and Accurate First Order Derivative Spectrophotometric Method have been developed for simultaneous estimation of Valsartan and Rosuvastatin calcium in Synthetic Mixture. Valsartan shows ZCP (Zero Crossing Point) at 250 nm and Rosuvastatin calcium show ZCP at 223 nm. Linearity Range of 8-40 µg/ml for Valsartan and 1-5 µg/ml for Rosuvastatin calcium with Correlation Coefficient of 0.9981 and 0.9972 Valsartan and Rosuvastatin calcium respectively. The Precision data obtained with less than 2% RSD. Accuracy was carried out by Recovery Studies and was obtained in the range of 98.80-98.58 % for Valsartan and 99.28-98.92% for Rosuvastatin calcium. The LOD and LOQ value were found to be 1.04 µg/ml, 3.16 µg/ml for Valsartan and 0.102 µg/ml, 0.309 µg/ml for Rosuvastatin calcium.

CONCLUSION:

A simple, accurate and precise first order derivative method has been developed and validated for routine analysis of Valsartan and Rosuvastatin calcium. The developed method is recommended for routine and quality control analysis of both the drugs in synthetic Mixture.

ACKNOWLEDGMENT:

The authors are highly thankful to Dr. K. Pundarikakshudu, Director of L. J. Institute of Pharmacy, Ahmedabad, India for providing valuable guidance and all the facilities to carry out the research work.

REFERENCES:

1. Ji-Yong Jang, Sang-Hak Lee, Byung Soo Kim, Hong Seog Seo, Woo-Shik,Youngkeun Ahn, Kwang Kon Koh, Tae-Soo Kang, Sang-Ho Jo, Bum-Kee Hong,, Hyoung-Yang.

Additive Beneficial Effects of Valsartan Combined with Rosuvastatin in the Treatment of Hypercholesterolemic Hypertensive Patients. Korean Circ J, 2015; 45(3): 225-233

2. Clinical Trail. Gov, "Fixed-dose Combination of Valsartan plus Rosuvastatin Versus Their Isolated Components for Hypertension and Dyslipidemia", November 2017, https://clinicaltrials.gov/ct2/show/NCT02662894
3. Pub Chem, "Valsartan", November 2017, https://pubchem.ncbi.nlm.nih.gov/compound/valsartan#section=Pharmacology
4. Pub Chem,"Rosuvastatin calcium", November 2017, https://pubchem.ncbi.nlm.nih.gov/compound/Rosuvastatin#section=Pharmacology-and-Biochemistry
5. Maryadele NJ. The Merck Index an Encyclopedia of chemicals drugs and biological. 14 th Edn., UK; Merck Research Laboratories: 2006, pp. 9916, 8270.
6. Indian Pharmacopoeia. Government of India Ministry of Health and Family Welfare Published by Indian Pharmacopoeia Commission, Ghaziabad, 2014, Vol-II.
7. Rowe RC, Sheskey PJ, Quinn ME. Handbook of Pharmaceutical Excipients. 6th Edn; Pharmaceutical press, London; 2009, pp. 210-214, 404-407, 663-666, 728-730.
8. Beckett AH, Stenlake JB, Practical Pharmaceutical Chemistry. 4th Edn; CBS Publication, 2001 part 1, pp 87.
9. ICH Q2 (R1), 2005, Validation of Analytical Procedure: Text and Methodology, International Conference on Harmonization, IFPMA, Geneva, Switzerland.