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Research Article

Method Development and Validation for Multicomponent Analysis of Rosuvastatin Calcium and Losartan Potassium in Bulk Drug by RP-HPLC

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ABSTRACT

A simple, sensitive and specific liquid chromatographic method was developed for the simultaneous estimation of Rosuvastatin calcium and Losartan potassium in bulk form. Chromatographic conditions included the Column C-18 (Shim-pack) 250 x 4.6 mm, particle size 5 µm, mobile phase acetonitrile, methanol and water pH 3 (orthophosphoric acid) in the ratio 20:25:55 recorded at 233 nm. The retention time were found to be 3.55 and 4.64 min and average percentage recovery 99.42% and 99.63% for Rosuvastatin calcium and Losartan potassium respectively. The proposed method was found to comply with ICH guidelines.

Keyword: Rosuvastatin calcium, Losartan potassium, RP-HPLC, accuracy and linearity.

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1. INTRODUCTION¹⁻¹⁰:

calcium (Fig 1) is a lipid-lowering drug that belongs to the statin class of medications. Used to lower the risk of cardiovascular disease and manage abnormal lipid levels. Rosuvastatin is a statin derivative and a competitive inhibitor of the enzyme HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase, which catalyzes the conversion of HMG-CoA to mevalonate which is early rate-limiting step in cholesterol biosynthesis.

Figure 1: Structure of Rosuvastatin calcium

Losartan (Fig 2) is an angiotensin II receptor blocker (ARB) used to treat hypertension. Losartan reversibly and competitively prevents angiotensin II binding to the AT₁ receptor in tissues like vascular smooth muscle and the adrenal gland. Losartan and its active metabolite bind the AT₁ receptor with 1000 times more affinity than they bind to the AT₂ receptor.

Figure 2: Structure of Losartan potassium

Literature review revealed only few efficient methods were available for quantitative estimation of the both the drugs. Hence, an attempt has been made to develop cost effective,

simple and precise RP-HPLC method to estimate both drugs in bulk drug. Quantitative analysis plays an important role in the development of new drugs and their commercial production as formulations. Pharmaceutical industries rely upon quantitative chemical analysis to ensure that the raw material used and the final products obtained meet the required specifications. So here an attempt has been made to develop simple, rapid and economic method for simultaneous estimation of Rosuvastatin calcium and Losartan potassium in bulk drug and formulation by using High Performance Liquid Chromatography.

2. EXPERIMENTAL WORK¹¹⁻¹⁶:

2.1 Chemicals and reagents

HPLC grade Acetonitrile, o-phosphoric acid, HPLC grade methanol, water, pH buffer capsule 4.0, 7.0, 9.2, phosphate buffer, THF, Rosuvastatin calcium and Losartan potassium.

2.2 Instrumentation: High Performance Liquid Chromatography (Shimadzu), manual sampler, software Win chrome and detector (UV-visible), Column C-18 (Shim-pack) 250 x 4.6 mm, particle size 5 μ m.

2.3 Preparation of stock solutions, working solutions and calibration standards

Standard Rosuvastatin calcium 100 mg was weighed and transferred to a 100 ml volumetric flask and dissolved in methanol. The flask was shaken and sonicate for 30 minute at 45°C and volume was made up to the mark with solvent. 10 ml of solution was pipetted out from this and transferred to 100 ml volumetric flask and the volume was made up to 100 ml with methanol. The conc. of prepared stock solution was 100 μ g/ml.

Standard Losartan potassium 100 mg was weighed and transferred to a 100 ml volumetric flask and dissolved in methanol. The flask was shaken and sonicate for 30 minute at 45°C and volume was made up to the mark with solvent. 10 ml of solution was pipetted out from this and transferred to 100 ml volumetric flask and the volume was made up to 100

ml with methanol. The conc. of prepared stock solution was 100 μ g/ml.

2.4 Selection of wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. For the selection of wavelength 10 μ g/mL concentration of Rosuvastatin calcium and 10 μ g/mL concentration of Losartan potassium was prepared in methanol and overlain spectra were obtained on UV spectrophotometer. The result of trials showed at 233 nm wavelength was most suitable for the analysis.

2.5 Selection of chromatographic condition

Chromatographic separation was achieved at ambient temperature on a reversed phase isocratic high performance liquid chromatography using a mobile phase consisting of methanol, acetonitrile and water by adjusting its pH 3 with orthophosphoric acid. The mobile phase was pumped at a rate of 1.0 mL/min. The detector wavelength was set at 235 nm and run time was 10 minutes.

To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase pH and flow rate were studied. The resulting chromatograms were recorded and the chromatographic responses were measured.

3. RESULT AND DISCUSSION

3.1 Optimized Method development¹⁷⁻¹⁹. The selection of the composition of mobile phase were studied and optimized. Separation was found to be satisfactory with acetonitrile, methanol and water pH 3 (orthophosphoric acid) in the ratio of 20:25:55 %, v/v. UV detection was carried out at 233 nm where both the drugs exhibit maximum absorption. Isocratic mode was chosen as the retention for both the drugs were less than 6 min at a flow rate of 1ml/min. Retention time for Rosuvastatin calcium and Losartan potassium with retention time of 3.55 and 4.64 min. The chromatogram of optimized trial is shown in Fig 3.

Figure 3: Optimized chromatogram of standard drug containing 10 μ g/ml Rosuvastatin calcium and 10 μ g/ml Losartan potassium at wavelength 233 nm

3.2 Validation of the developed method

3.2.1 Linearity curve for the Rosuvastatin calcium²⁰⁻²²:

From the standard stock solution of Rosuvastatin calcium (100 µg/mL) 2, 4, 6, 8 and 10 were pipetted out and transferred to separate 10 ml of volumetric flasks and the volume was made up to 10 ml with the help of mobile phase.

These concentrations were of 20, 40, 60, 80 and 100 µg/ml respectively. The injection was given at time interval of 10 minutes with run time of 10 minutes. The linearity was obtained in selected conc. ranges. Linearity of Rosuvastatin calcium is shown in Table 1 and calibration plot in Fig 4.

Table 1: Linearity curve of Rosuvastatin calcium at wavelength 233 nm.

Sr. No	Conc. (µg/mL)	Area (µ volt sec.)
1	20	1548754
2	40	3198757
3	60	4658794
4	80	6352489
5	100	8125481

Figure 4: Calibration plot of Rosuvastatin calcium at 233nm

3.2.2 Linearity curve for the Losartan Potassium:

For Losartan potassium from standard stock solution (100 µg/mL) 1.5, 3, 4.5, 6 and 7.5 ml were pipetted out and transferred to separate 10 ml of volumetric flasks and the

volume was made up to mark respectively with solvent. These concentrations were of 15, 30, 45, 60 and 75 µg/ml respectively. The linearity was obtained in selected conc. ranges. Linearity of Rosuvastatin calcium is shown in Table 2 and calibration plot in Fig 5.

Table 2: linearity curve of Losartan Potassium at wavelength 233 nm.

Sr. No	Conc. (µg/mL)	Area (µ volt sec.)
1	15	1310158
2	30	2548455
3	45	4015878
4	60	5287945
5	75	6475485

Figure 5: Linearity of Losartan potassium at 233nm

3.2.3 Accuracy²³⁻²⁶

Accuracy is the closeness of the test results obtained by the method to the true value. Accuracy may often express in terms of percent recovery of assay of known amount of analyte added. Recovery studies were carried out by addition

of standard drug to the sample at 3 different levels of spiking i.e. 80%, 100% and 120% of the actual amount taking into consideration percentage purity of added bulk drug sample. Accuracy of Rosuvastatin calcium and Losartan potassium are shown in table 3 & 4 respectively.

Table 3: Accuracy of Rosuvastatin calcium at wavelength 233 nm.

Sr. No.	Amt. of sample Taken	Amt. of drug added	Level of addition (%)	Amount Recovered µg/mL			Mean	S.D	% RSD	% Drug recovery
	(µg/mL)	(µg/mL)		1	2	3				
1	20	16	80%	35.73	35.75	35.77	35.75	0.020	0.055	99.79
2	20	20	100%	39.82	39.76	39.95	39.84	0.097	0.243	99.87
3	20	24	120%	43.74	43.80	43.83	43.79	0.045	0.102	99.88
Mean of % RSD									0.133	99.42
Mean of % Drug Recovery										

Table 4: Accuracy of Losartan potassium at wavelength 233 nm.

Sr. No.	Amt. of sample Taken	Amt. of drug added	Level of addition (%)	Amount Recovered µg/mL			Mean	S.D	% RSD	% Drug recovery
	(µg/mL)	(µg/mL)		1	2	3				
1	30	24	80%	53.70	53.85	53.74	53.76	0.077	0.143	99.55
2	30	30	100%	59.77	59.91	59.68	59.78	0.115	0.193	99.63
3	30	36	120%	65.89	65.80	65.75	65.81	0.070	0.107	99.71
Mean of % RSD									0.147	99.63
Mean of % Drug Recovery										

3.2.4 Precision²⁷⁻²⁹

The precision of an analytical method is the degree closeness of agreement between a series of measurements obtained from the multiple sampling of the same sample. Precision include repeatability, inter and intraday precision and reproducibility.

3.2.4 Interday & intraday precision

Interday & intraday precision of conc. 40, 60, 80 µg/mL was prepared and data was obtained for Rosuvastatin calcium. 3

Replicates were prepared for 3 days. Intraday and Inter day precision of Rosuvastatin calcium are shown in table 5 & 7 respectively.

Interday & intraday precision of conc. 30, 45, 60 µg/mL was prepared and data was obtained for Losartan potassium. 3 replicates were prepared for 3 days. The absorbance for intraday were measured in 2 hours of interval table 6 & 8 respectively.

Table 5: Intraday precision of Rosuvastatin calcium at wavelength 233 nm.

Conc.	40 µg/mL	60 µg/mL	80 µg/mL
Area (µ volt sec.)	3198754	4658794	6352489
	3196722	4657025	6352750
	3193688	4658411	6354875
Mean	3196328	4658077	6353371
S D	2449.46	930.68	1308.74
% RSD	0.079	0.0019	0.020

Table 6: Intraday precision of Losartan potassium at wavelength 233 nm.

Conc.	30 µg/mL	45 µg/mL	60 µg/mL
Area (µ volt sec.)	2548455	4015878	5287945
	2546489	4015002	5286128
	2543441	4016855	5287369
Mean	2546128	4015912	5287147
S D	2426.38	926.95	928.56
% RSD	0.099	0.023	0.017

Table 7: Interday precision of Rosuvastatin calcium at wavelength 233nm

Conc.	40 µg/mL	60 µg/mL	80 µg/mL
Absorbance	3254821	4492973	6068210
	3256887	4494808	6063307
	3254096	4493926	6067274
Mean	3255268	4493902	6066264
S D	1448.19	917.72	2602.7
% RSD	0.044	0.020	0.042

Table 8: Interday precision of Losartan potassium at wavelength 233 nm.

Conc.	30 µg/mL	45 µg/mL	60 µg/mL
Area (µ volt sec.)	2739221	4472437	5848712
	2737392	4474480	5845732
	2738348	4475451	5846696
Mean	2738320	4474123	5847047
S D	914.81	1538.44	1563.20
% RSD	0.033	0.034	0.026

3.2.5 Repeatability³⁰

For repeatability minimum of 6 determinants were prepared of 60 µg/mL conc. and the chromatogram responses were

obtained by injecting one by one. The standard deviation & relative standard deviation was calculated for each type of precision. Repeatability of Rosuvastatin calcium and Losartan potassium are shown in table 9 & 10 respectively.

Table 9: Repeatability of Rosuvastatin calcium at wavelength 233nm

Sr. No	Area (µ volt sec.)
1	3198754
2	3196722
3	3193688
4	3196328
5	3197856
6	3192544
Mean	3195982
S D	2406.05
%RSD	0.075

Table 10: Repeatability of Losartan potassium at wavelength 233nm

Sr. No	Area (µ volt sec.)
1	2548455
2	2546489
3	2543441
4	2546128
5	2545658
6	2546322
Mean	25466082
S D	1613.14
%RSD	0.063

3.2.6 Limit of detection (LOD)³¹⁻³²

LOD is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOD was calculated by the standard deviation of the response and the slope.

$$LOD = \frac{3.3 \times \sigma}{S}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope and standard deviations were calculated from the linearity curve obtained for conc. ranges of 20-100 $\mu\text{g/mL}$ for Rosuvastatin calcium and 15-75 $\mu\text{g/mL}$ for Losartan potassium.

3.2.7 Limit of quantitation (LOQ)³³⁻³⁵

It is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and

accuracy. LOQ was calculated by the standard deviation of the response and the slope. The data was obtained from linearity curve and the LOQ was calculated.

$$LOQ = \frac{10 \times \sigma}{S}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope and standard deviations were calculated from the linearity curve obtained for conc. ranges of 20-100 $\mu\text{g/mL}$ for Rosuvastatin calcium and 15-75 $\mu\text{g/mL}$ for Losartan potassium.

LOD & LOQ for Rosuvastatin calcium and Losartan potassium are shown in table 11 & 12 respectively.

Table 11: LOD & LOQ of Rosuvastatin calcium at 233 nm wavelength

Conc.	Abs.	Amount Recovered $\mu\text{g/mL}$	% Drug recovery
20	1548754	20.40883781	102.0442
40	3198757	40.64533458	101.6133
60	4658794	58.55198931	97.58665
80	6352489	79.32434753	99.15543
100	8125481	101.0692455	101.0692
Mean			100.2938
SD			1.872892
SE of Intercept			102144.2
SD Of Intercept			228401.4
LOD			9.244071
LOQ			28.01233

Table 12 : LOD & LOQ of Losartan potassium at 233 nm wavelength

Conc.	Abs.	Amount Recovered $\mu\text{g/mL}$	% Drug recovery
15	1310158	14.96106	99.7404
30	2548455	29.17247	97.24157
45	4015878	46.01346	102.2521
60	5287945	60.61244	101.0207
75	6475485	74.24133	98.98844
Mean			99.84865
SD			1.916969
SE of Intercept			86118.71
SD Of Intercept			192567.3
LOD			7.293043
LOQ			22.10013

3.2.8 Robustness ³⁶⁻³⁹

Robustness is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameter. For HPLC robustness was carried out by changing wavelength

and flow rate. Robustness of a method was done by change in wavelength, or change in flow rate of a mobile phase. Injection of 20 µg/mL was prepared from the stock solution and the recorded. Robustness data for Rosuvastatin calcium and Losartan potassium are shown in Table 13 and 14.

Change in Wavelength

Table 13: Robustness of Rosuvastatin calcium and Losartan potassium at wavelength 233±2 nm.

Wavelength	Difference	Rosuvastatin calcium (min.)	Losartan potassium (min.)
231	-2	3.465	4.464
233	0	3.550	4.358
235	+2	3.598	4.129

Table 14: Robustness of Rosuvastatin calcium and Losartan potassium at wavelength 233nm by changing the flow rate.

Flow rate (ml/min.)	Difference	Rosuvastatin calcium (min.)	Losartan potassium (min.)
0.9	3.466	4.421	3.662
1	3.550	4.358	3.550
1.1	3.602	4.375	3.453

The overall validation parameter data are shown in Table 15. It complied with ICH guidelines.

Table 15: Summary of validation parameters of RP-HPLC at 233nm wavelength.

Parameter	Rosuvastatin calcium	Losartan potassium
Linear range in (µg/mL)	20-100	15-75
Regression coefficient (R ²)	0.9989	0.9988
%Accuracy	99.42	99.63
Repeatability (n=6)	%RSD NMT 2	%RSD NMT 2
Precision		
Interday precision	%RSD NMT 2	%RSD NMT 2
Intraday precision		
LOD	9.244071	7.293043
LOQ	28.01233	22.10013

4. DISCUSSION

Reversed phase-high performance liquid chromatography (RP-HPLC)

the method was developed for the simultaneous estimation of Rosuvastatin calcium and Losartan potassium using Reverse Phase – High Performance Liquid Chromatography. All the parameters were validated according to the ICH guidelines and meet all the limits. Various trials were taken for the Rosuvastatin calcium and Losartan potassium at certain conditions. The linearity for method was obtained at 233 nm for Rosuvastatin calcium and Losartan potassium. The R² values were found 0.9988 and 0.9989 for Rosuvastatin calcium and Losartan potassium respectively. The R² value was within the limits of 0.995- 0.999, and has

good linearity. For accuracy of method the % drug recovery was calculated for both drugs at wavelength 233 nm at conc. of 80, 100, 120% and average recovery was found 99.42 % for Rosuvastatin calcium and 99.63 % for Losartan potassium. As the % drug recovery should be considered within limits i.e. 100 ± 2%. Interday precision was calculated by preparing dilutions of 40, 60 and 80 µg/ml conc. for Rosuvastatin calcium and 30, 45, 60 µg/ml concentrations for Losartan potassium & responses were obtained at wavelength 233nm. Interday precision for Rosuvastatin calcium for 1st day the %RSD was found 0.079, 0.019, and 0.020 at wavelength 233. For 2nd day the %RSD was found 0.044, 0.020, and 0.042 at wavelength 233 nm. For 3rd day the %RSD was found 0.049, 0.039, and 0.036 at wavelength 233 nm. The concentration 40, 60 and 80 µg/ml was used in triplicate at different days. Interday precision for Losartan

potassium for 1st day the %RSD was found 0.033, 0.034, and 0.026 at wavelength 233. For 2nd day the %RSD was found 0.049, 0.036, and 0.036 at wavelength 233 nm. For 3rd day the %RSD was found 0.063, 0.035, and 0.037 at wavelength 233 nm. The concentration 30, 45, 60 µg/ml was used in triplicate at different days. For repeatability the % RSD was found 0.075 % at wavelength 233 nm for Rosuvastatin calcium. For Losartan potassium the % RSD was found 0.063 % at wavelength 233 i.e. within the limit. It shows that the method qualifies the criteria of repeatability. LOD & LOQ was calculated from the linearity curve at wavelength 233 and the LOD was found 9.2440 µg/ml and LOQ was found 28.0123 µg/ml for Rosuvastatin calcium. For Losartan potassium LOD was found 7.2930 µg/ml and LOQ was found 22.1001 µg/ml at wavelength 233 nm. Robustness of Rosuvastatin calcium and Losartan potassium at 233 nm

The method was found robust as there was change in wavelength to ± 2 mL and change in flow rate of ± 0.1 mL/min.

5. CONCLUSION:

RP-HPLC method was developed for Rosuvastatin calcium and Losartan potassium. The chromatographic condition for optimized method was found to consisting of Column C-18 (Shim-pack) 250 x 4.6 mm, particle size 5 µm, mobile phase acetonitrile, methanol and water pH 3 (orthophosphoric acid) in the ratio 20:25:55. The retention time were found to be 3.55 and 4.64 min and average percentage recovery 99.42% and 99.63% for Rosuvastatin calcium and Losartan potassium respectively. The proposed methods were found to comply with ICH guidelines. These methods can be further employed in future for the routine determination of Rosuvastatin calcium and Losartan potassium in bulk drug.

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