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Simultaneous Determination of Atorvastatin Calcium and Losartan potassium in bulk and combined dosage forms by validated RP-HPLC with UV detection

Devi Ramesh[1], Mohammad Habibuddin*[2], Rajendra Narayan Dash[2],Touseef Humaira[2]
1. Government Polytechnic for women, Gujarathipeta, Srikakulam, Andhra Pradesh, India.
2.Adept Pharma & Bioscience Excellence Private Limited, Corporate Office: 10-2-289/26,Road No.2 Shanti Nagar, Hyderabad, Pin: 500028,Andhra Pradesh, , Indian.

ABSTRACT

A simple, rapid, and precise RP-HPLC method for simultaneous analysis of Losartan potassium and Atorvastatin calcium in bulk and its pharmaceutical formulations has been developed and validated. Atorvastatin was separated from losartan by using Grace Smart Altima C-18 column (25 cm × 4.6 mm, 5-µm) with a mobile phase consisting of acetonitrile: 10mM phosphate buffer (55:45 %v/v, pH 3.0) a flow rate of 1mL/min and detection wavelength at 240 nm. Aceclofenac was used as an internal standard in this method. Losartan, atorvastatin and aceclofenac were eluted with retention times of 4.85 min, 8.31min and 9.51 min respectively. The method was validated for accuracy, precision, linearity and sensitivity in accordance with ICH (Q2B) guidelines and the results of all the validation parameters were found to be within the acceptable limits. The calibration plots were linear over the concentration ranges from 200-30000ng/mL (r^2 = 0.999) for both the drugs. Accuracy and precision were determined by QC sample covering low, medium, and high concentration levels. Intra and inert-day accuracy were found to be 97.16-102.57% for losartan and 97.01-103.05% for atorvastatin. The limit of quantification was found to be166ng/mL and 179ng/mL for losartan and atorvastain respectively. The method was successfully applied for the assay of the dosage form, recovery of the individual drugs from the combined tablet dosage was found to be >97% for both the drugs. From the results it is suggested that the proposed method is simple, reproducible, accurate and precise.

Keywords: Aceclofenac, Atorvastatin, Losartan, RP-HPLC, Simultaneous estimation, Validation.

*Corresponding Author Email: drhabib21@gmail.com
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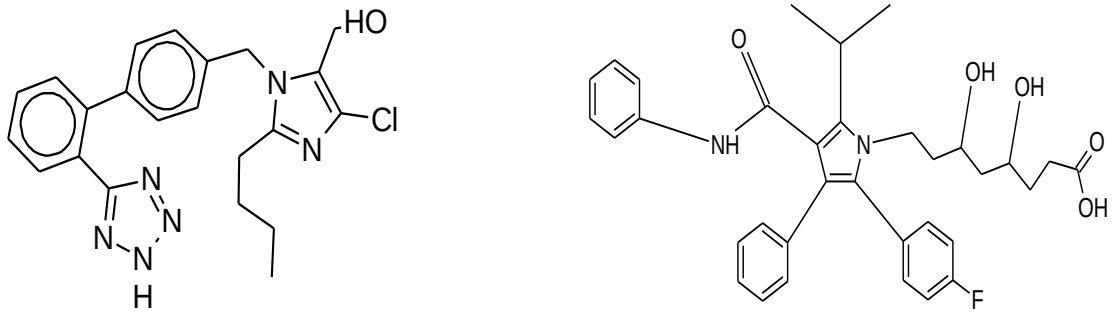
INTRODUCTION

Losartan potassium (LOS) is a white to off-white free-flowing crystalline powderand it is a derivative of imidazole-5-acetic acid attenuate vasoconstriction induced by Ang II. LOS is the first orally active antagonist of the AT1-receptor subtype1 and used for the treatment of hypertension[1]. Chemically, Losartan [Figure. 1(a)] is2-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-methanol mono potassium salt[2]. LOS and its principal active metabolite block the vasoconstriction and aldosterone-secreting effects of angiotensin II. Losartan selectively blocks the binding of angiotensin II to the AT_1receptor found in many tissues[3]. Atorvastatin calcium (ATR) is a synthetic hydroxyl methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor that has been used as a lipid lowering agent[4]. Chemically, ATR [Figure. 1(b)]is [R-(R*, R*)]-2-(4-flurophenyl)-B, B—dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H-pyrrole-1-heptanoic acid[5]. ATR is a competitive inhibitor of HMG-CoA reductase. This enzyme catalyzes the reduction of 3-hydroxy-3-methylgultaryl-coenzyme-A to mevalonate, which is the rate-determining step in the hepatic cholesterol synthesis. Because cholesterol synthesis decreases, hepatic cells increase the number of LDL receptors on the surface of the cells, which in turn increase the amount of LDL uptake by the hepatic cells, and decrease the amount of LDL in the blood [6,7].



(a) (b)

Figure. 1. Chemical structures of (a) Losartan potassium (b) Atorvastatin calcium

The literature survey revealed that no HPLC methods are reported for the simultaneous determination of LOS and ATR till date. Methods are available for the quantification of LOS individually and with other combinations other than ATR[8-11]. Methods are available for the quantification of ATR individually and with other combinations other than LOS[12-16]. Earlier we developed simple UV methods for simultaneous determination ATR and LOS from formulation[17]. Present study involves the development and validation of a RP-HPLC method for the simultaneous determination of LOS and ATR in bulk and its pharmaceutical formulations.

MATERIALS AND METHODS

Instrumentation

The instruments employed in this study were as follows; HPLC- Analytical Technologies, UV-2230 detector and 2230-pump with A2000 Software, Gujarat, India. Centrifuge apparatus- Remi, Mumbai, India. Sonicator-Remi, Mumbai, India. Analytical balance-Sartorius, German. Millipore Direct-Q 3 UV Barnstead Thermolyne, USA. pH meter-Systronics, Ahmadabad, India.

Standards and chemicals

LOS, ATR and Aceclofenac (ACF) were gifted samples obtained from S. L. Drugs (Hyderabad, India). Purified water was prepared using a Millipore Direct-Q 3 U.V with the pump water purification system. Acetonitrile, methanol of HPLC grade, orthophosphoric acid, sodium dihydrogen phosphate were purchased from Merck Ltd. (Mumbai, India).

Stock and working solution preparation

Preparation of standard stock solution:

Accurately weighed and transferred 100mg of LOS and ATR into a 100mL volumetric flask dissolved and made up of the volume with methanol. Daily working standard solutions of LOS and ATR was prepared by suitable dilution of the stock solution with the mobile phase.

Preparation of a buffer:

10mM strength of phosphate buffer is prepared by weighing 680mg of potassium dihydrogen phosphate in 500ml HPLC grade water adjusted to pH 3 by using orthophosphoric acid.

Method validation

The validation parameters like linearity, sensitivity, accuracy, precision, specificity, and stability, according to the ICH guidelines were carried [18].

Selectivity is studied by comparing the chromatograms obtained from placebo sample and tablet sample. Calibration curves are prepared by assaying standard samples contains two drugs, ranging from 200-30000 ng/mL. The linearity of the method was determined by plotting the ratio of peak area of drug to peak area of IS (y) versus the nominal concentration (x) of drug, respectively. The calibration curves are constructed by least squares linear regression.

Intra- and inter-day accuracy and precision of the method was determined at three different concentration levels on 3 different days, and on each day, three replicates were analyzed with independently prepared calibration curves. The accuracy and precision were expressed as percentage of accuracy and the relative standard deviation (%RSD) respectively and calculated by using equation (1) and (2).

$$\text{Accuracy (\%)} = \frac{\text{Mean observed concentration}}{\text{Nominal concentration}} \times 100 \quad \text{\_\_\_\_\_Eq.(1)}$$

$$\text{\% RSD} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100 \quad \text{\_\_\_\_\_Eq. (2)}$$

The limit of detection (LOD) and limit of quantification (LOQ) are defined as the lowest concentration giving a signal-to-noise ratio of at least 3-fold and 10-fold, respectively. The LOD and LOQ of this method were verified based on the standard deviation of response and slope by using the equations (3) and (4).

$$\text{LOD} = \frac{3.3\sigma}{\text{slope}} \ldots\ldots\ldots \text{Eq. (3)}$$

$$\text{LOQ} = \frac{10\ \sigma}{\text{slope}} \ldots\ldots\ldots \text{Eq. (4)}$$

Where; σ = Standard deviation of intercept from the calibration curve

Slope = Average slope of the calibration curve

The stability of the drug solution was determined for the short-term by keeping at room temperature (25°C) for 24h to check the degradation of drugs during the analysis and freeze-thaw stability was studied by keeping the samples in freeze for (2-8 ^{o}C) for 24h and thawed at room temperature (25 ^{o}C) for 24h. This was repeated for two more cycles and analyzed. Each sample injected three times into HPLC and concentrations obtained were compared with the nominal values of the QC samples.

Analysis of dosage form

Twenty Tablets (ZIVAST-LFORTE, FDC Pvt. Ltd) were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to one tablet (10mg of ATR and 50mg of LOS)was transferred into 100mL volumetric flask, then added 40mg of the ATR pure drug and drugs were extracted with methanol. This solution was filtered through What Mann No.1 filter paper and the solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined. The sample was injected (n=5) into the HPLC. The amount of each drug recovered was calculated from the respective linearity graph.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

Method optimization:

LOs and ATR are hydrophobic, almost insoluble in aqueous solutions and are freely soluble in

methanol; the reverse-phase chromatography was adopted. Hydrophobic C-18 a stationary phase column was tried. During the method development, top priority was given for the complete separation of LOS, ATR. The chromatographic method was optimized by changing various parameters, such as the pH of the mobile phase, organic modifier and buffer used in the mobile phase and the composition of the mobile phase. Buffers like Ammonium acetate, phosphate buffer in various strengths are tried along with methanol and acetonitrile as organic solvent. A Mixture of acetonitrile and phosphate buffer (pH 3.0) (in the proportions of 45:55, 50:50, 65:45, 60:40, 65:35, 80:20, 70:30(%v/v), were tested as a mobile phase with Grace Smart C-18 column. The mobile phase composition of 55:45 %v/v acetonitrile: buffer was given good resolution, retention time with minimal tailing factor in acceptable range. The method was optimized with the mobile phase composition of acetonitrile and phosphate buffer 55:45(%v/v).

We investigated several compounds to find a suitable IS, by preparing a reference standard solution containing analyte i.e. LOS and ATR along with Aceclofenac (ACF) in the above selected mobile phase. It was injected six times on to HPLC and observed the peak shape, response and interference of these peaks with the analyte. Then chose ACF, as an internal standard in this study, it is producing good peak shape, reproducible results and no interference with analyte peak is observed.

Buffer molarity of 10, 20 and 50 mM was tested. There were no significant changes in the chromatographic response and peak shape with change in buffer molarity. A buffer molarity of 10 mM was selected for further analysis.

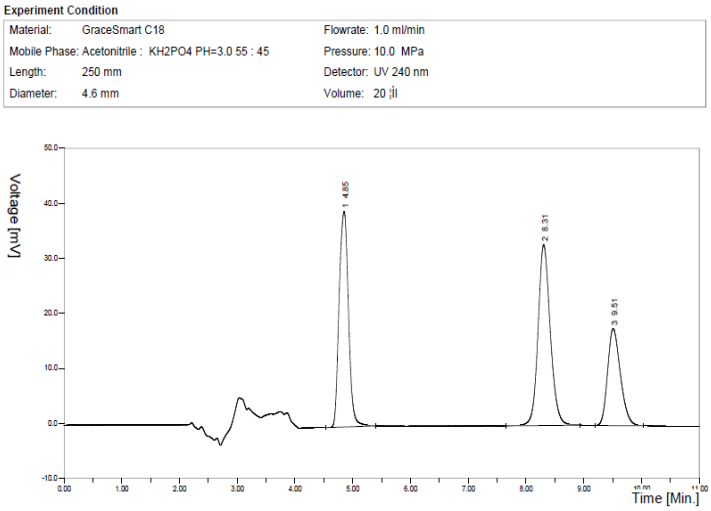


Figure 2. Standard chromatogram of LOS (4.85 min) and ATR (8.31 min), ACF (9.51min)

After several trials, the method was optimized as a mixture of 10mM potassium dihydrogen phosphate buffer (pH 3.0) and acetonitrile (45:55 %v/v), at a flow rate of 1mL/min, at 240nm for

run time 12min. These chromatographic conditions achieved satisfactory resolution, retention time and tailing for both drugs of LOS and ATR along with internal standard (ACF). The Figure-2 shows that standard chromatogram of LOS and ATR and these are well separated from each other.

System suitability:

To check the system suitability, working stock standard of individual drugs was injected into the HPLC to determine the individual retention times of drugs. Then working standard mixture solution was injected five times and retention time, tailing factor(T), resolution(Rs) and theoretical plates(N) were observed. Then calculated the percentage relative standard deviation (%RSD) for five consecutive injections for each parameter. These parameters for this method were found to be within the acceptable limits. The limit of the resolution between two adjacent peaks should be a =2 and tailing factor should be ≤219 and the %RSD should be ≤2. System suitability parameters of this method were presented in [Table. 1]. System suitability test confirmed that the chromatographic system was adequate for the analysis planned to be done.

Table.1. System suitability parameters of ATR and LOS

Parameters	**LOS** Mean±SD, RSD	**ATR** Mean±SD, RSD	**ACF** Mean±SD, RSD	**Required limits**
RT in minites (R_t)	4.85±0.014, 0.29	8.31±0.03, 0.36	9.51±0.02, 0.27	RSD≤2
Theoretical plates (N)	16839±117, 0.70	31743±254, 0.80	35681±169, 0.47	N>2000
Tailing Factor (T)	1.21±0.01, 1.24	1.17±0.02, 1.82	1.13±1.82	T≤2
Resolution(R_s)		10.26±0.11, 1.07	4.16±0.04, 0.96	R_s>2

Values are expressed in Mean±SD, n=5

METHOD VALIDATION

Selectivity:

The selectivity of the present method is established by checking the blank sample and observed the chromatogram. There is no interference found at retention times of LOS and ATR in the blanks concludes the selectivity of the method. The carryover effect of the present method was established by using six injections of blank and an upper limit of quantification (ULOQ) of LOS and ATR. These samples were analyzed alternately to check any carryover in the blank sample. In this study there were no such effects observed.

Linearity:

The linearity of this method is evaluated by linear regression analysis, which is calculated by the least square method and the drug is linear in the concentration range of 200-30000 ng/mL for both drugs. Calibration standards are prepared by spiking required volume of working standard

(100µg/mL) solution and 100µl of IS from 500µg/mL solution into different 10 mL volumetric flasks and volume made up with methanol to yield concentrations of 200, 500, 1000, 2000 and 5000, 10000 and 30000 ng/mL of mixture. The resultant peak area of each drug was measured. Calibration curve is plotted between the ratios of drug to IS peak areas against concentration of the drug. The (Figure 3) shows the linearity graph regression coefficient (r^2) including the slope and y-intercept. This linearity (200-30000ng/mL) will cover all the strengths of LOS and ATR.

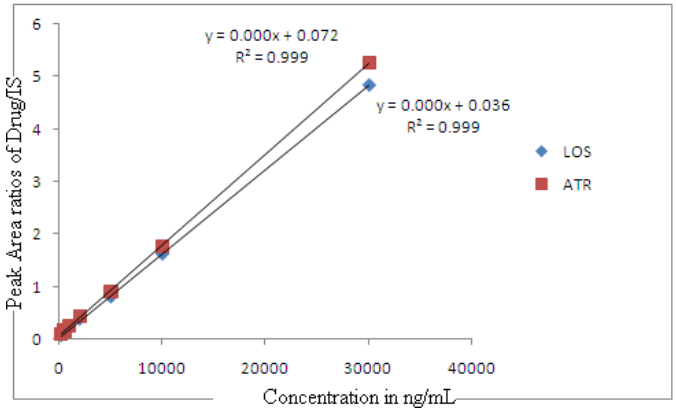


Figure 3. Linearity graph of LOS and ATR

Sensitivity:

The limit of detection (LOD) and limit of quantification (LOQ) are defined as the lowest concentration giving a signal-to-noise ratio of at least 3-fold and 10-fold, respectively. The LOD and LOQ of this method were verified based on the standard deviation of response and slope The LOD and LOQ were found to be 54ng/mL, 166ng/mL for LOS and 59ng/mL, 179ng/mL for ATR respectively.

Intra-day and Inter-day Precision and Accuracy:

The intra- and inter-day precision and accuracy of this method is determined by analyzing replicates of QC samples at three concentrations in 3 different days. The coefficients of variation for the intra- and inter-day precision were <3%. The intra- and inter-day accuracies were found to be more than 97% for both drugs. The low levels of coefficients of variation (Table.2), indicate the method is accurate and precise.

Table.2. Intra and Inter-day accuracy and precision of ATR and LOS

Conc. (µg/mL)	Intra-day (n=6)				Inter-day (n=9)			
	LOS		ATR		LOS		ATR	
	mean±SD	RSD (%)	mean±SD	RSD (%)	mean±SD	RSD (%)	mean±SD	RSD (%)
0.3	102.38±3.7	3.65	103.02±2.7	2.66	102.57±1.4	1.44	103.05±2.1	2.13
8	99.08±3.89	3.92	98.45±0.89	0.90	103.87±1.2	1.21	98.29±0.89	0.91
20	97.79±0.73	0.75	97.84±0.06	0.06	97.16±0.57	0.58	97.01±0.96	0.99

Values expressed Mean±SD

Robustness and Ruggedness:

Robustness of the method was done by changing slight variation in the parameters like mobile phase composition, flow rate and wavelength. Present method didn't show any significant change when the critical parameters were modified. The tailing factor for both the drugs was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature may conclude that the method conditions were robust. Ruggedness is studied along with precision and accuracy of batches where the effects of column and analyst change are observed. The observed value for column variation and results obtained for precision and accuracy are within the acceptable criteria (i.e. There are no changes in the retention time, recovery and precision of the drug) according to ICH[18].

Stabilities:

The stability of the drug was studied at different conditions for quality control (QC) of samples. The samples were analyzed and compared with freshly analyzed QC samples and the accuracy was found to be 97-103% for both drugs in two conditions, concludes that there was no degradation of drugs during the analysis.Table.3 represents the stability data of two drugs.

Table.3. Auto sampler and short-term stability of Atorvastatin and Losartan (n=3)

Conc.(µg/mL)	Freeze-Thaw stability				Short-term stability			
	LOS		ATR		LOS		ATR	
	mean±SD	RSD (%)	mean±SD	RSD (%)	mean±SD	RSD (%)	mean±SD	RSD (%)
0.3	96.42±1.14	1.18	101.87±2.8	2.81	96.52±1.25	1.30	99.05±0.14	0.14
8	103.46±1.4	1.37	98.46±0.55	0.56	101.34±1.5	1.52	97.75±1.85	1.89
20	99.37±0.78	0.79	98.11±0.46	0.47	99.80±0.70	0.70	98.26±0.50	0.51

Values expressed Mean±SD

Application of method for analysis of dosage form

The method was successfully applied to the marketed formulation and the percentage recovery was found to be more than 97% for both drugs. The amount and recovery of each drug for the assay of formulations were represented in (Table. 4). The recovery was found to be> 97% indicates that the excipients used in pharmaceutical formulation were not interfering with the recovery of drugs. The chromatogram from the formulation was shown in (Figure.4).

Table.4. Recovery study from formulation (n=3) of Losartan and Atorvastatin

Brand Name	Labeled amount(mg)		Calculated amount (mg) ± SD		Assay (%)	
	ATR	LOS	ATR	LOS	ATR	LOS
ZIVAST-LFORTE	10	50	9.791±0.013	49.313±0.42	97.91	98.62

Values are expressed in Mean ±SD, n=3

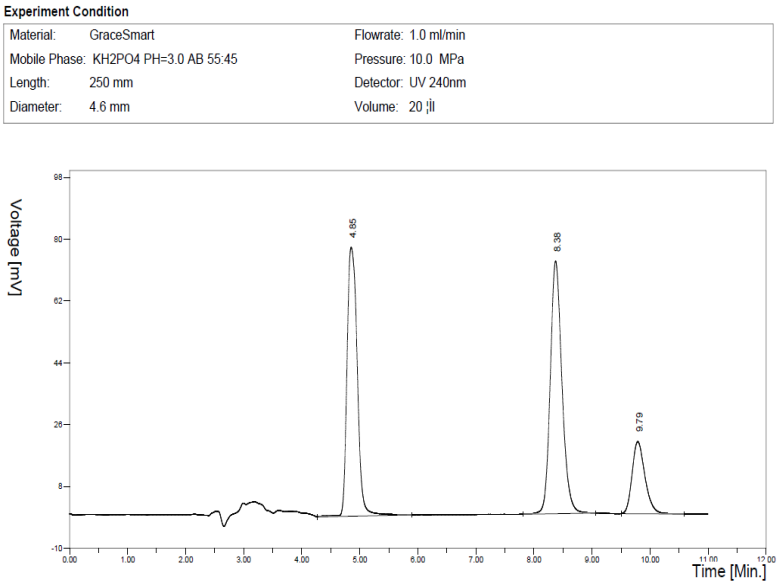


Figure 4. Chromatogram of LOS and ATR from formulation

CONCLUSIONS:

It can be seen from the results and discussion presented above; the proposed method has good sensitivity, and is Specific, Precise and Robust. The results of the analysis of pharmaceutical formulations reveal that the proposed method is suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. The proposed method is simple, sensitive, reliable, and can be used for the simultaneous determination of Atorvastatin and Losartan in Pharmaceutical formulation and in pure drug.

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