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A NEW RS HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF IMPURITIES IN LOSARTAN POTASSIUM IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT: Losartan is used for hypertension, including in people with left ventricular hypertrophy (enlarged heart muscle), and kidney dysfunction among type II diabetics. It may also delay progression of diabetic nephropathy. It is a suitable pharmacological agent for the reduction of renal disease progression in patients with type 2 diabetes, hypertension, and microalbuminuria (>30 mg/24 hours). A Related Substance High Performance Liquid Chromatography method has been developed and validated for Losartan Potassium. Chromatographic separation of the pharmaceutical was performed on an Inert Sil ODS 3V column (5µm 4.6 mm×250 mm) with a 250:50:2 (v/v) mixture of acetonitrile, methanol and Triethylamineas mobile phase, at a flow rate of 1.0 mL min^{-1}, and detection at 237 nm. Separation was complete in less than 20 min. The method was validated for linearity, accuracy, precision, limit of quantitation, and robustness. Linearity, accuracy, and precision were found to be acceptable over the ranges 50–150.00 g mL^{-1} for Losartan Potassium.

INTRODUCTION: Drug combinations are single preparations containing active pharmaceutical ingredients (APIs) for the purpose of their concurrent administration as a drug. Single dose toxicity studies in relevant species are helpful in identifying the upper bounds of exposure or effects on target organs. The concern for overdosing is much less for biopharmaceuticals in large part due to their method of delivery. The end points that are measured are generally, more extensive especially when larger animal species are used.

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However, monographs in most official pharmacopoeia are for single component drugs, hence local Pharmaceutical manufacturing companies in the analysis of single drug formulations use methods that involve multiple and repeated extractions to extract each active component before their quantification using spectrophotometry or titrimetry. Such methods are thus laborious and cumbersome. This has led to researchers developing various methods to help facilitate easy and quick analysis of multi-component drugs.

With HPLC being a method of choice, many researchers have worked at developing various RP-HPLC methods for the simultaneous estimation of various active components in single drugs. Losartan potassium is an orally active, nonpeptide angiotensin II (AII) receptor antagonist [1].

It is the first of a new class of drugs to be introduced for clinical use in hypertension [2]. Losartan is a medicine called angiotensin receptor blocker (ARB). It is chemically described as 2-butyl-4-chloro - 1[p - (o - 1H -tetrazol-5-ylphenyl)-benzyl] imidazol-5-thanol monopotassium salt. Losartan potassium is a first-line drug for the treatment of hypertension and as such, is included in clinical guidelines for antihypertensive treatment in countries with high health surveillance it is also part of the model list of essential medicines of the World Health Organization (WHO) for its proven efficacy and safety [3]. The potassium salt of losartan is well absorbed orally with a systemic bioavailability of approximately 33%. Losartan potassium is very well tolerated. In clinical trials, dizziness was the only drug-related event reported more frequently with losartan potassium monotherapy than with placebo [4]. First-dose hypotension is uncommon. An aspect of the drug tolerability profile which may prove to be particularly advantageous is that it is associated with a similar incidence of cough to placebo in patients with a history of ACE inhibitor- related cough [5]. Additionally, clinically relevant adverse metabolic effects or laboratory abnormalities have not been documented during losartan potassium therapy and renal function is preserved in patients with or without renal insufficiency [6].

According to European pharmacopoeia (According to European pharmacopoeia (Ph. Eur.) 7.0 , there are eleven related impurities and degradation products of losartan, from that we are estimated and validated degrading impurities like Impurity J ([2-butyl - 4 - chloro - 1-[[2- (1H – tetrazol – 5 - yl) biphenyl-4-yl] methyl]-1H-imidazol-5-yl] methyl acetate) as LS1 , Impurity K ((2-butyl-4-chloro-1-[[2′ -(1H-tetrazol5-yl) biphenyl-4-yl] methyl]-1H-imidazol-5-carbaldehyde) as LS2 and impurity L ([2-butyl-1-[[2′ -[1-[[2-butyl-4-chloro-1-[[2′ -(1H-tetrazol-5- yl)biphenyl-4-yl]methyl]-1H-imidazol-5-yl]methyl] - 1Htetrazol – 5 - yl] biphenyl - 4-yl]methyl]-4-chloro-1H-imidazol-5- yl]methanol) as LS3 by HPLC [7].

MATERIALS AND METHODS: Fixed dose combination tablet Losara- 50 was purchased from local market, Goregaon, Maharashtra, India. Acetonitrile, Triethylamine and Orthophosphoric acid used were HPLC grade, purchased from Merck. Chemicals. All dilutions were performed in standard volumetric flasks.

Instrumentation and Chromatographic Conditions: Chromatography was performed with a Shimadzu high-performance liquid chromatography comprising a LC20AD pump, equipped with 20-µL loop, and a Shimadzu SPD20A detector. A double-beam spectrophotometer was use for scanning and selecting the detection wavelength [7]. Chromatograms and data were recorded by means of Lab Solutions software. An Inert Sil ODS 3V column (5µm 4.6 mm×250mm) was used for the analysis. The mobile phase was a 250:50:2 (v/v) mixture of acetonitrile, methanol and Triethylamine in isocratic mode with column oven temperature maintained at 30°C and elution monitored by a UV detector at 237 nm. All noted measurements were performed with an injection volume of 20 µl of samples dissolved in a diluent consist of a 250:50:2 (v/v) mixture of acetonitrile, methanol and Triethylamine. During development of bio-analytical procedure, diluent was changed accordingly.

Buffer Solution: Place 385 mg of ammonium acetate to a 1000ml volumetric flask, dissolve in water, dilute to volume with water, and mix. Filter the obtained solution before use.

Mobile Phase A: Prepare a blend solution of 700 ml of a buffer solution, 250 ml of acetonitrile, 50 ml of methanol, and 2ml of triethylamine. Adjust the solution pH to 6.60 with glacial acetic acid.

Preparation of Losartan Standard Stock Solution: Accurately weighed 12.55mg losartan potassium standard to a 50mL volumetric flask, dissolve in the mobile phase, and dilute to volume with the mobile phase.

Preparation of LS1 Impurity (Impurity J) Stock Solution: Accurately weighed 6.10 mg LS1 impurity standard to a 100mL volumetric flask, dissolve in the mobile phase and dilute to volume with the mobile phase.

Preparation of LS2 Impurity (Impurity K) Stock Solution: Accurately weighed 6.09 mg LS2 impurity standard to a 100mL volumetric flask, dissolve in the mobile phase, and dilute to volume with the mobile phase.

Preparation of LS3 Impurity (Impurity L) Stock Solution: Accurately weighed 6.11 mg LS3 impurity standard to a 100mL volumetric flask, dissolve in the mobile phase and dilute to volume with the mobile phase.

Mix Standard Solution: Place 1mL of the losartan Standard stock solution and 2.5ml of each impurity standard stock solution to a 50mL volumetric flask, dilute to volume with the mobile phase, and mix.

Placebo Preparation for 50 mg: Placed 5 tablets to a 250mL volumetric flask, added about 150mL of the mobile phase, and sonicated until homogeneous dispersion. Diluted to volume with the mobile phase. Filtered the solution through a 0.45µm membrane filter.

Test Solution 50 mg: Placed 5 tablets to a 250mL volumetric flask, added about 150mL of the mobile phase, and sonicated until homogeneous dispersion. Diluted to volume with the mobile phase. Filtered the solution through a 0.45µm membrane filter.

Spiked Sample Preparation for 50 mg: Placed 10 tablets to a 250mL volumetric flask, added about 150mL of the mobile Phase and sonicated until homogeneous dispersion. Add 12.5ml of LS1 impurity stock solution12.5ml LS2 impurity stock solution and12.5 ml LS3 impurity stock solution. Diluted to volume with the mobile phase. Filter the solution through a 0.45µm membrane filter.

Method Validation: The proposed method was validated according to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines Q2 (R1) [8].

System Suitability [9]: System Suitability Test (SST) is generally performed to evaluate the suitability and effectiveness of the entire chromatographic system not only prior to use but also during the time of analysis. The system suitability experiment was carried out before the determination of all active substances in unknown sample. The coefficient of variation was less than 2 % for replicate measurements of the same sample.

Specificity: Specificity is the ability of the analytical method to distinguish between the analyte(s) and the other components in the sample matrix. The specificity experiment was carried out there was no interference of placebo peak with standard.

Linearity: Linearity of the method was studied by injecting seven concentrations of the drug prepared in the diluent in triplicate into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. For peak shapes refer **Fig. 5, 6**.

The plots were linear for Losartan in the range 1 to 7.50 µg/mL. **Fig 1.** The data were analyzed by linear regression least-squares fitting. The statistical data obtained is given in **Table 1.**

TABLE 1: ANALYSIS PERFORMANCE DATA OF LOSARTAN

	Losartan
Linear working range	1 to 7.50 µg/mL
Slope	63642.4553
Intercept	-20562.8613
Correlation coefficient	0.999

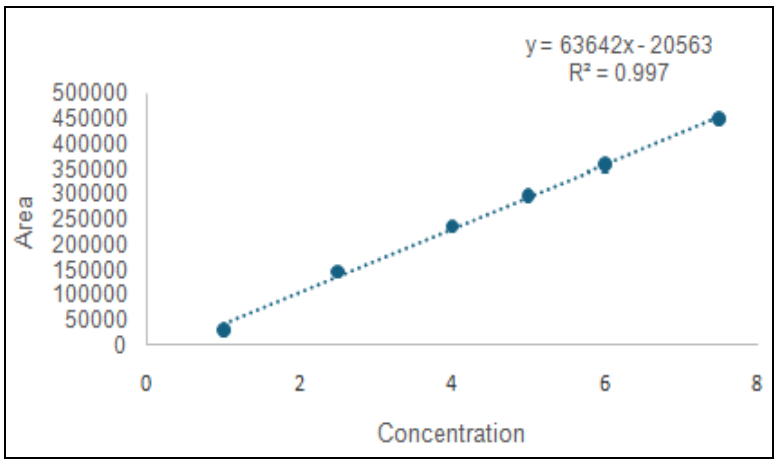

FIG. 1: LINEARITY PLOT FOR LOSARTAN POTASSIUM

Accuracy: Accuracy was determined by applying the method to synthetic mixtures of drug product components to which known quantities of each drug substance corresponding to 50%, 100%, and 150% of the label claim of each drug was added. The accuracy was expressed as the percentage of analytes recovered by the assay. Mean recoveries for Losartan from the specific formulations are shown in **Table 2**. The results indicate the method is highly accurate for simultaneous determination of four drugs.

TABLE 2: ACCURACY OF LOSARTAN

Accuracy Level		Losartan
50	Mean	100.31
100		100.32
150		99.53
50	SD	0.811
100		0.464
150		0.052
50	% RSD	0.81
100		0.46
150		0.05

Precision: The precision of an analytical method is a measure of random error and is defined as the agreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (%CV) or relative standard deviation (RSD) of the replicate measurements. The precision of the method was demonstrated by inter day and intraday variation studies. In the inter day and intraday studies, Six replicate injections of the mixed standard solutions and sample solutions were made and the percentage RSD were calculated. From the data obtained, the developed HPLC method was found to be precise. Refer **Table 3, Table 4** and **Table 5.**

TABLE 3: ASSAY RESULTS OF METHOD PRECISION

Sample	Losartan (% Assay)
Sample-1	99.096
Sample-2	99.102
Sample-3	99.109
Sample-4	99.098
Sample-5	99.096
Sample-6	99.102
Mean	99.101
STD DEV	0.005
% RSD	0.01

TABLE 4: ASSAY RESULTS OF INTERMEDIATE PRECISION FOR LOSARTAN

Sample	Losartan (% Assay)
Sample-1	99.097
Sample-2	99.097
Sample-3	99.103
Sample-4	99.083
Sample-5	99.086
Sample-6	99.093
Mean	99.093
STD DEV	0.007
% RSD	0.01

TABLE 5: % RSD FOR 12 SAMPLES OF LOSARTAN

Sample	Losartan (% Assay)
Sample-1	99.096
Sample-2	99.102
Sample-3	99.109
Sample-4	99.098
Sample-5	99.096
Sample-6	99.102
Sample-7	99.097
Sample-8	99.097
Sample-9	99.103
Sample-10	99.083
Sample-11	99.086
Sample-12	99.093
Mean	99.097
STD DEV	0.007
% RSD	0.01

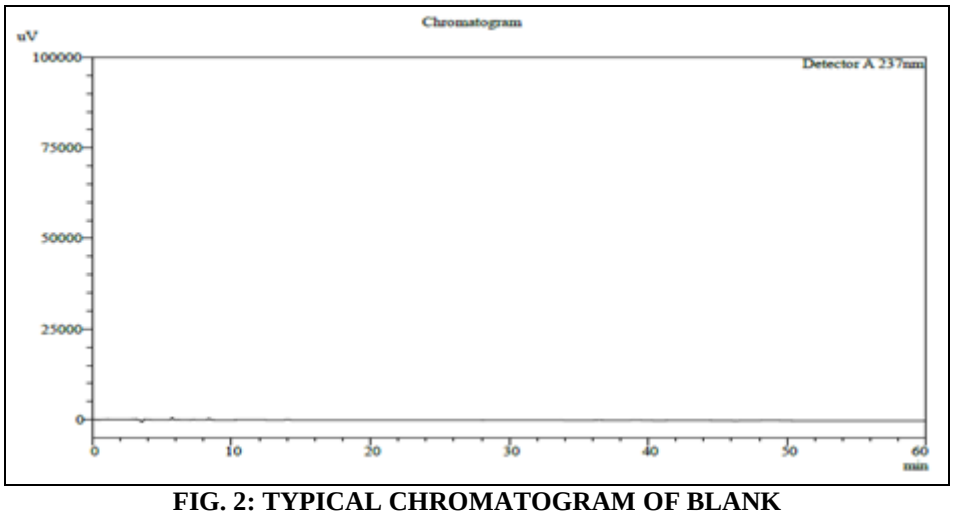

FIG. 2: TYPICAL CHROMATOGRAM OF BLANK

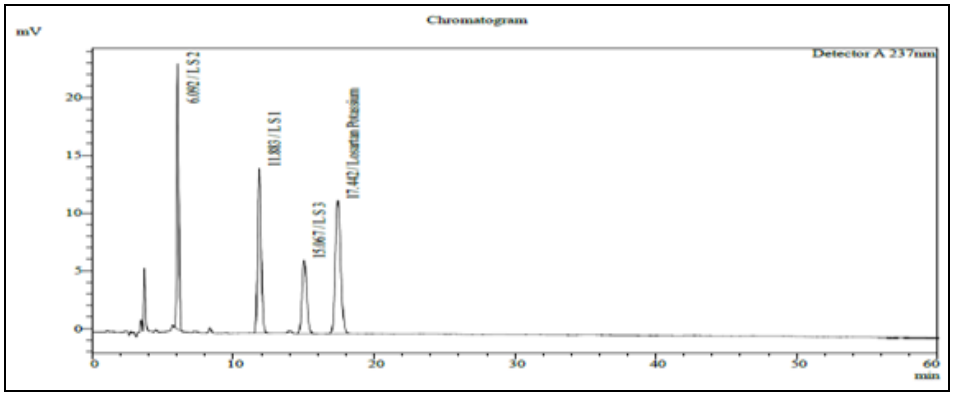

FIG. 3: TYPICAL CHROMATOGRAM OF STANDARD

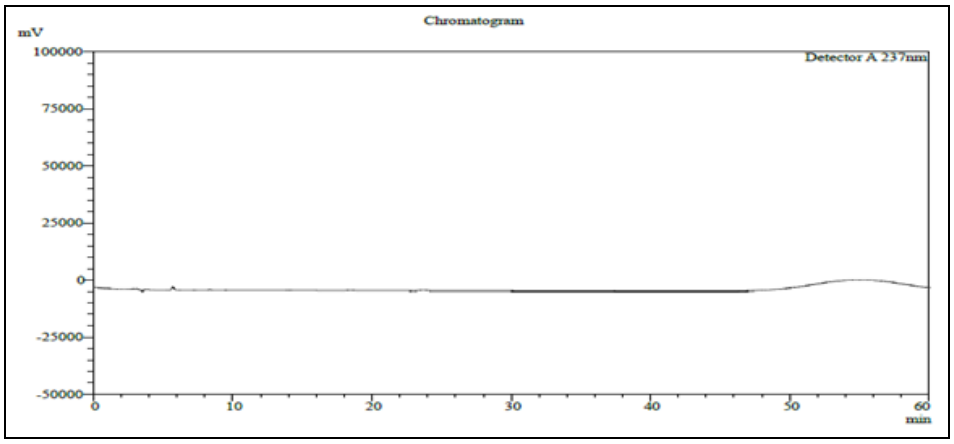

FIG. 4: TYPICAL CHROMATOGRAM OF PLACEBO 50 mg

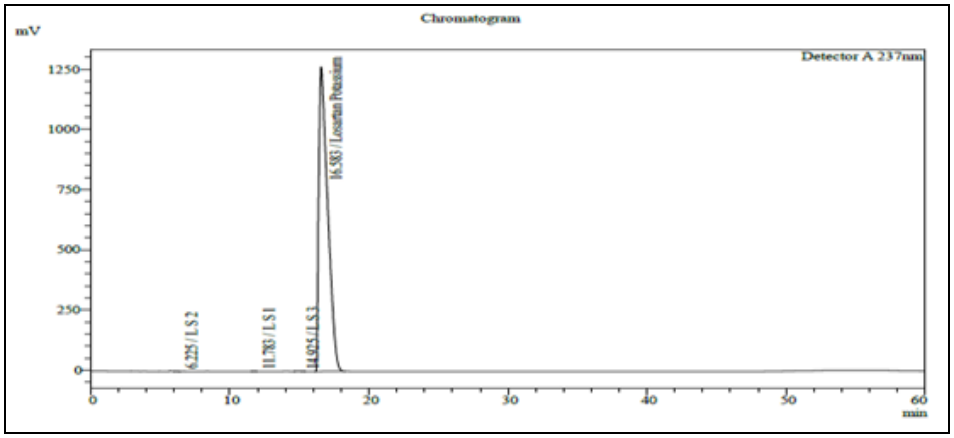

FIG. 5: TYPICAL CHROMATOGRAM OF SAMPLE 50 mg

Stability Studies: The stability experiments were aimed at testing all possible conditions that the samples might experience after collecting and prior the analysis. The stability of the drug spiked for short term bench top (at room temperature for 0 h, 1 h, 2 h, 4 h, 8 h, 12 h, 16 h and 24 h), were evaluated. Stability of all analytes in analytical solution was observed at room temperature for period of 24 h.

Robustness: The Robustness of the proposed method was evaluated by altering the temperature of the column oven and by variation in the flow rate. The Flow rate of the Sample was changed by ±2 ml (0.9 ml and 1.1 ml) and the wavelength was changed by ±2 nm (235 nm and 239 nm). At both the ends of the column oven temperature variations and change in flow rate, there was no significant difference in the peak resolution and retention time of Losartan. The results indicated that the separation of two active substances is achievable under the given conditions using the method developed, which is satisfactory for the simultaneous determination of Losartan in tablet dosage form.

RESULTS AND DISCUSSION: Use of Inert Sil ODS 3V column (5µm 4.6 mm×250 mm) with mobile phase 250:50:2 (v/v) mixture of acetonitrile, methanol and Triethylamine as mobile phase in isocratic mode resulted in good separation of two drug substances and the reference standards. Regression analysis of the calibration data for Losartan showed that the dependent variable (peak area) and the independent variable (concentration) were represented by the equation Y = 63642x - 20563 for Losartan respectively. The system suitability experiment was carried out before the determination of all active substances in an unknown sample. The coefficient of variation was less than 2 % for replicate measurements of the same sample. This shows that the method and the system both are suitable for the determination of unknown sample. Precision studies including the instrument precision, intra-assay precision and intermediate precision was carried out to evaluate the precision of the method. The intermediate precision included analysis on a different day and by a different analyst. The values of standard deviation and coefficient of variation were calculated. The coefficient of variation for Losartan for intra-assay precision was 0.01and for inter-day precision 0.01 respectively. The low values of standard deviation and coefficient of variation indicate high precision of the method. The results from recovery analysis are given in Table 2.0. The robustness experiment was carried out by altering the flow rate and wavelength by (±2 ml and ±2 nm) respectively. The mean recovery is within acceptable limits, indicating the method is accurate.

CONCLUSION: The proposed method is highly specific, accurate, selective, precise, and reproducible and can therefore be used for a routine quality-control analysis and quantitative simultaneous determination of Losartan in pharmaceutical preparations.

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CONFLICT OF INTEREST: The authors confirm that there are no conflicts of interest to declare.

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