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A NEW RP-HPLC METHOD FOR SIMULTANEOUS ASSAY OF LOSARTAN POTASSIUM AND AMLODIPINE PURE AND IN PHARMACEUTICAL FORMULATION

N.MURALI KRISHNA,[*1] BV SREENIVASULU,[2] S.V.M. VARDHAN[3]

[1]Department of Science and humanities, VR.Siddhartha Engineering College, Kanuru, Vijayawada.

[2]Department of Biochemistry, Acharya Nagarjuna University,Guntur,AP,India

[3]Department of Biochemistry, Krishna University P.G.Centre,Nuzvid,AP,India

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ABSTRACT

A simple, precise cost effective and RP-HPLC method has been developed and validated for the simultaneous determination of losartan potassium and amlodipine in pure and pharmaceutical formulations. Chromatographic separation of ciprofloxacin and tinidazole were made on Aligent, Zorbax C18 column using 0.01M sodium dihydrogen phosphate buffer (pH 4.0) and acetonitrile in the ratio of 600:400 v/v as mobile phase at a flow rate of 1.0ml/min and the detection was carried out at 225nm. The linearity range was found to be 300-900µg/ml for losartan potassium and 30-90µg/ml for amlodipine and the detection was made at 225nm. The proposed method was validated as per the ICH guidelines.

KEYWORDS: Losartan potassium and amlodipine, RP-HPLC and Validation.

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INTRODUCTION

Losartan Potassium (LSP)[FIG.1.A], 2-n-butyl-4-chloro-1-[p-(o-1Htetrazol- 5-ylphenyl) benzyl]-imidazole-5 methanol monopotassium salt is a highly selective, orally active, non-peptide angiotensin II receptor antagonist indicated for the treatment of hypertension. It has a more potent active metabolite (II, 2-n-butyl-4-chloro-1-[2- (1H-tetrazol-5 yl) biphenyl- 4-yl) methyl] imidazole-5- carboxyl acid)[1]. The determination of Losartan has been carried out in tablets by HPLC, capillary electrophoresis and super-critical fluid chromatography[2,3], in urine by gas chromatography- mass spectrometry[4] and, simultaneously with its active metabolite in biological fluids, by HPLC[5- 10].

Amlodipine Besylate (ADB))[FIG.1.B], chemically, 2-[(2- aminoethoxy) methyl]- 4- (2-chlorophenyl) -1, 4- dihydro- 6-methyl-3, 5-pyridinedicarboxylic acid 3-ethyl.5-methyl ester, is an anti-hypertensive and an antianginal agent in the form of the besylate salt, Amlodipine besylate. It is not official in any Pharmacopoeia.

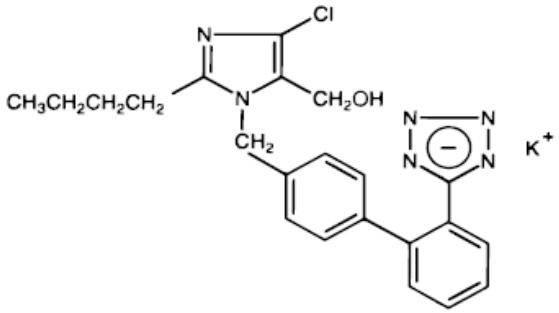

Fig .1.A.Structure of Losartan Potassium

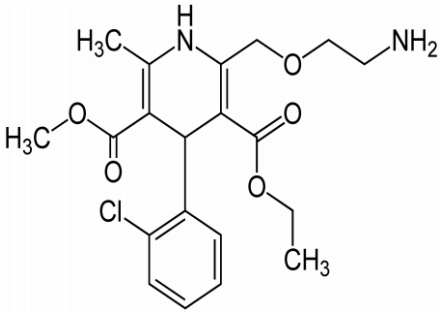

Fig.1.B.Structure of Amlodipine Besylate

Various analytical methods have been reported for the assay of Amlodipine besylate[11] in pure form as well as in pharmaceutical formulations that include high performance liquid chromatography,[12-17] reversed phase high performance liquid chromatography,[18-21] high performance thin layer chromatography,[22-25] gas chromatography,[26] gas chromatography–mass spectrometry,[27] liquid chromatography with tandem mass spectrometry[28] and fluorimetry,[29] derivative spectroscopy,[30,31] simultaneous multicomponent mode of analysis and difference spectrophotometry[32-34]. In the present paper an attempt have been by the author made in developing a new RP-HPLC method for the assay of Losartan potassium and Amlodipine and in combined dosage form and was validated following ICH guidelines.

MATERIALS AND METHODS

1. CHEMICALS AND SOLVENTS: Pharmaceutical grade Losartan potassium and Amlodipine were kindly supplied as a gift sample by Dr.Reddy's Laboratory, Hyderabad, India. Acetonitrile and methanol of HPLC grade and sodium dihydrogen phosphate of AR grade was used in the present assay. Water HPLC grade was obtained from a Milli-QRO water purification system.

Losartan potassium and Amlodipine of purity (995) was obtained as gifted sample form Hetero Drugs Ltd,Hyderabad. Formulations of Losartan potassium and Amlodipine available in the market as Losar-A (Unichem pharmaceuticals, Himachal pradesh, India.) of composition of Losartan potassium (100mg) Amlodipine (10mg) were purchased and used in the present assay.

2.INSTRUMENTAL AND ANALYTICAL CONDITIONS: The HPLC analysis of losartan potassium and amlodipine was carried out on a waters LC system equipped with 2695pump, 2996 photodiode array detector and Aligent Zorbax-C18 column (250 mmx4.6 mm I.D; particle size 5µm) which was procured from Waters Corporation, Ireland. The output of signal was monitored and integrated using waters Empower 2 software. The injection volume of sample was 5µL. An isocratic mobile phase containing 0.01M sodium dihydrogen phosphate buffer (pH 4.0) and acetonitrile in the ratio of 600:400 v/v was carried out with the flow rate of 1.0mL/min at ambient column temperature. Before the analysis, the mobile phase was degassed and filtered through a 0.45µm membrane filter. The photodiode array UV-detector was set to a wavelength of 225nm for the detection and chromatographic runtime was 10minutes. The entire HPLC system was equilibrated before making each injection.

3. BUFFER PREPARATION: Accurately weigh and transfer about 2.72gms of Sodium dihydrogen phosphate (monohydrate) and 2.0mL of triethylamine in 1000mL of purified water and mix. Adjust pH to 4.0 (±0.05) with dilute orthophosphoric acid solution. Filter the solution through 0.45µm membrane filter.

4. MOBILE PHASE PREPARATION: Prepare a filtered and degassed mixture of buffer (pH 5.0) and acetonitrile in the ratio of 600:400 v/v was used as mobile phase in current assay respectively.

5. DILUENT PREPARATION: Methanol is used initially as diluents for extracting the drug and consequent dilutions are made with mobile phase.

6. PREPARATION OF STANDARD SOLUTION: Standard stock solutions containing Losartan Potassium (LSP) and Amlodipine besylate (ADB) were prepared individually by dissolving 100 mg of LSP and quantity of ADB equivalent to Amlodipine base 10mg separately in 20 ml of methanol. It was then sonicated for 10 minutes and

the final volumes of both the solutions were made up to 100ml with methanol. From this stock solution working solutions containing 300-900µg/mL of Losartan Potassium and 30-90µg/ mL of Amlodipine besylate were prepared by using the same diluent respectively.

7. ANALYSIS OF MARKETED SAMPLE (DOSAGE FORMS): A total of 20 tablets were accurately weighted and triturated with glass mortar and pestle. An amount equivalent to one tablet (containing 5 mg of LP and 0.5 mg of AB) was transferred to a 100ml volumetric flask; 50 ml of mobile phase was added and the flask was kept in an ultrasonic bath for 10 min. The volume was made up to mark and the solution was filtered through 0.2 micron nylon membrane filter. The final volumes of both the solutions were made up to 100 ml with mobile phase. From this solution sample solution containing 300-900µg/ mL of Losartan Potassium and 30-90µg/ mL of Amlodipine besylate were prepared in the same way as described in preparing working standard solutions respectively. 20µL of these diluted solutions were injected to the column and were analyzed under the described optimized chromatographic conditions

RESULTS & DISCUSSION

A. METHOD DEVELOPMENT: To develop a suitable and robust LC assay method for losartan potassium and amlodipine, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Aligent,Zorbax column (250 mmx4.6 mm I.D; particle size 5µm) with the following mobile phase of degassed mixture of buffer [pH - 4.0] and acetonitrile in the ratio of 500:500 v/v. Losartan potassium and amlodipine peaks was eluted at void volume respectively. For next trial the mobile phase composition was changed slightly. The mobile phase composition was buffer and acetonitrile in the ratio of 550:450 v/v. The above trail resulted in the little broad peak shape with long retention time. Again the mobile phase composition changed slightly to buffer and acetonitrile in the ratio of 600:400 v/v respectively with the eluent at flow rate set at 1.0mL/min. In this trail losartan potassium and amlodipine eluted with a retention time of 2.051&3.249 minutes resulting in sharp peak which is detected at 221 and 226nm and the validative chromatogram of losartan potassium and amlodipine standard using the proposed method is represented in (Fig.2). Losartan potassium and amlodipine showed a significant UV absorbance at wavelength 225nm and hence, this wavelength has been chosen for detection in analysis of losartan potassium and amlodipine respectively. The system suitability results of the developed RP-HPLC method are presented in Table.1.

B.METHOD VALIDATION: The developed RP-LC method extensively validated for assay of losartan potassium and amlodipine using the following parameters.

1.SPECIFICITY:

A. BLANK AND PLACEBO INTERFERENCE: A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution showed no peaks at the retention time of losartan potassium and amlodipine. This indicates that the diluent solution used in sample preparation do not interfere in estimation of losartan potassium and amlodipine in their formulations tablets. Similarly chromatogram of placebo solution showed no peaks at the retention time of losartan potassium and amlodipine peaks. This indicates that the placebo used in sample preparation do not interfere in estimation of losartan potassium and amlodipine in dosage form.

2.SYSTEM SUITABILITY: System suitability is an integral part of chromatographic system. At first the HPLC system was stabilized for 40 min. One blank followed by six replicate analysis of solution containing 100% target concentration of losartan potassium and amlodipine were injected to check the system suitability. To ascertain the system suitability for the proposed method, a number of parameters such as theoretical plates, retention time were taken and results along with optimized chromatographic conditions were presented in Table.1.

TABLE 1: System suitability parameters for Losartan Potassium And Amlodipine by the proposed RP-HPLC method

NAME OF THE COMPOUND	THEORETICAL PLATES	TAILING FACTOR
LOSARTAN POTASSIUM	6603	1.079
AMLODIPINE	6349	1.160

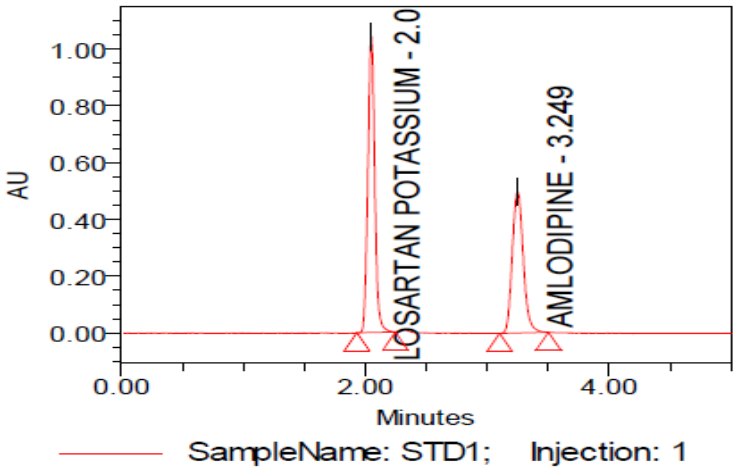

Figure: 2- Validated chromatogram of losartan potassium and amlodipine standard with the proposed method

3. LINEARITY & DETECTOR RESPONSE(LOD&LOQ): Replicate analysis of solution containing 300-900µg/ml and 30-90µg/ml of losartan potassium and amlodipine sample solutions respectively were injected into HPLC according to the procedure in a sequence and chromatograms were recorded. Calibration curves were constructed by plotting by taking concentrations on X-axis and ratio of peak areas of standards on Y-axis (Figs.3.A&B) and regression equation were computed for both drugs and represented in Table.2. The LOD and LOQ of losartan potassium and amlodipine was found to be 2.941, 2.903µg mL^{-1} and 9.80,9.675µg mL^{-1} respectively.

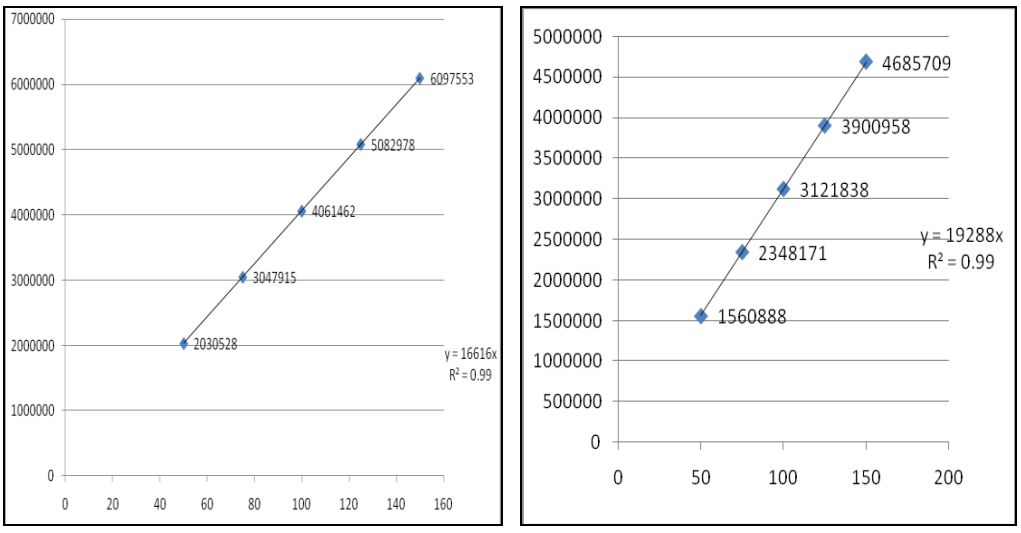

Figure: 3A&B - LINEARITY CURVE FOR LOSARTAN POTASSIUM AND AMLODIPINE

TABLE: 2 Linearity studies of Losartan potassium and Amlodipine by the proposed method

LINEARITY OF RESPONSE FOR LOSARTAN POTASSIUM AND AMLODIPINE				
% LEVEL (APPROX.)	LOSARTAN POTASSIUM		AMLODIPINE	
	CONCENTRATION (µg/mL)	PEAK AREA	CONCENTRATION (µg/mL)	PEAK AREA
50	300	2030528	30	1560888
75	450.00	3047915	45	2348171
100	600.00	4061462	60	3121838
125	750	5082978	75	3900958
150	900	6097553	90.00	4685709
Slope			16616	19288
RSQ			0.9995	0.999
LOD (µg/mL)			2.94	2.903
LOQ (µg/mL)			9.80	9.67

4.PRECISION: Precision study of sample (Losartan potassium and Amlodipine) was carried out by estimating corresponding responses 6 times on the same day for the 100% target concentration. The percent relative standard deviation (%RSD) is calculated which is within the acceptable criteria of not more than 2.0. Results of the above precision studies of Losartan potassium and Amlodipine are summarized in Table:3.

Table 3: Precision (inter and intraday) studies for Losartan potassium and Amlodipine by the proposed method

S No	Name	LOSARTAN POTASSIUM Area	AMLODIPINE Area
1	Injection-1	4069225	3125419
2	Injection-2	4065009	3122467
3	Injection-3	4060415	3124063
4	Injection-4	4066766	3120106
5	Injection-5	4063659	3124233
6	Injection-6	4066559	3120496
Avg		4065272	3122727
Std Dev		3025.756	2153.356
% RSD		0.074	0.068

5. ACCURACY: To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard losartan potassium and amlodipine were added to pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies are shown in Table.4 respectively.

Table 4: RECOVERY STUDIES FOR LOSARTAN POTASSIUM AND AMLODIPINE BY THE PROPOSED METHOD

LOSARTAN POTASSIUM						
Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% Recovery	% Mean
50%	445.65	2030511	297.400	294.57	99	
50%	445.65	2033448	297.400	295.00	99	
50%	445.65	2033360	297.400	294.98	99	99
50%	445.65	2037578	297.400	295.60	99	
50%	445.65	2037268	297.400	295.55	99	
50%	445.65	2030834	297.400	294.62	99	
100%	891.30	4067261	594.800	590.05	99	
100%	891.30	4061646	594.800	589.23	99	99
100%	891.30	4067760	594.800	590.12	99	
150%	1337.00	6090388	892.234	883.55	99	
150%	1337.00	6097102	892.234	884.52	99	
150%	1337.00	6095283	892.234	884.26	99	99
150%	1337.00	6090260	892.234	883.53	99	
150%	1337.00	6093445	892.234	883.99	99	
150%	1337.00	6096057	892.234	884.37	99	

AMLODIPINE						
Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% Recovery	% Mean
50%	445.65	1565530	29.920	29.91	100	
50%	445.65	1564559	29.920	29.89	100	
50%	445.65	1562622	29.920	29.85	100	100
50%	445.65	1563962	29.920	29.88	100	
50%	445.65	1568356	29.920	29.96	100	
50%	445.65	1566308	29.920	29.92	100	
100%	891.30	3128661.00	59.840	59.77	100	
100%	891.30	3126493.00	59.840	59.73	100	100
100%	891.30	3127916.00	59.840	59.76	100	
150%	1337.00	4681986	89.764	89.45	100	
150%	1337.00	4682015	89.764	89.45	100	
150%	1337.00	4688800	89.764	89.58	100	100
150%	1337.00	4686699	89.764	89.54	100	
150%	1337.00	4685157	89.764	89.51	100	
150%	1337.00	4684297	89.764	89.49	100	

6.ROBUSTNESS & RUGGEDNESS: To evaluate the robustness, the developed method was subjected to small deliberate variations in the optimized method parameters like variation of column temperature (35^{o}C and 37^{o}C). Standard solutions of losartan potassium and amlodipine was injected in replicate under varied chromatographic conditions and the standard deviation of the retention time of each analyte were calculated. The developed RP-HPLC method was found to be robust as the slight deliberate variation in temperature did not lead to changes in retention times.

The ruggedness of the method was determined by carrying out the experiment on Shimadzu HPLC instrument by two different analysts (I & II) using the same column. It was observed that there were no

marked changes in the chromatograms, demonstrating the RP-HPLC method developed by the author is rugged.

7. ASSAY IN FORMULATIONS: For the analysis of pharmaceutical formulations an amount of the powder equivalent to 25mg was accurately weighed, transferred into a 100 ml volumetric flask, dissolved in 70ml of mobile phase, sonicated, make up to the volume with mobile phase and filtered through 0.45µm membrane filter. The solution obtained was diluted further with the mobile phase so as to obtain concentrations in the range of linearity previously determined for the pure drug. The sample solution was injected under the chromatographic conditions and the chromatogram was recorded. The results of the formulation assay by the developed method were shown in Table.5.

TABLE.5: ESTIMATION OF AMOUNT LOSARTAN POTASSIUM AND AMLODIPINE PRESENT IN FORMULATIONS

Formulation	Labeled Amount	*Recovered Amount	% Recovery
LOSARTAN POTASSIUM	100mg	99.99mg	99.99
AMLODIPINE	10mg	9.93mg	99.93

*Average of six determinations

CONCLUSION

In conclusion, the RP-HPLC method described enabled the quantification of losartan potassium and amlodipine in pure and combined tablet dosage form. The results of validation studies demonstrated the good precision and accuracy, which proved the reliability of the proposed RP-HPLC method for the quantitative estimation of both the above said drugs in oral dosage forms.

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