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RP-HPLC method development and validation for simultaneous estimation of Losartan Potassium, Amlodipine Besilate and Hydrochlorthiazide in tablet dosage form

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Abstract

A high-performance liquid chromatographic method has been developed for the simultaneous analysis of Losartan potassium, Amlodipine besilate and Hydrochlorthiazide in combined solid dosage form. The mobile phase consisting of phosphate buffer (pH 7.0), methanol and acetonitrile in ratio of 60:20:20% v/v was delivered at the flow rate of 1.0 mL/min and UV detection was carried out at 238nm. The separation was achieved using C18 reverse-phase column (250 X 4.6 mm I.D., particle size 5µm). The method was linear over the concentration range of 200.24-300.36 µg/mL for Losartan potassium and 27.84-41.76 µg/mL for Amlodipine besilate and 50.00-75.00 µg/mL for Hydrichlorthiazide.The analytical recovery obtained was 100.15%. The validation of method carried out as per ICH guidelines. The described HPLC method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form and can be employed for bioequivalence study in future for the same formulations.

Keywords:- RP-HPLC, Losartan potassium, Amlodipine besilate, Hydrochlorthiazide, Validation.

INTRODUCTION

Amlodipine besilate (AML), chemically, [3-ethyl-5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-methyl-1-dihydropyridine-3,5-dicarboxylate benzenesulfonate [(1)] , is a long acting calcium channel blocker used which is used as an antihypertensive agent [1,2,3,4] .

Losartan Potassium (LP), 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[2,5,6] . Hydrochlorthiazide is 6-Chloro–3,4–dihydro–2*H*-1,2,4–benzothiadiazine–7–sulfonamide1,1–dioxide,used for the treatment of hypertension.Molecular formula is $C_7H_8ClN_3O_4S_2$ [2,5,6].

Literature survey revealed that a number of methods have been reported for estimation of Losartan Potassium, Amlodipine Besilate and Hydrochlorthiazide individually or in combination with other drugs [7-11]. However, there is no analytical method reported for the simultaneous estimation of Losartan Potassium, Amlodipine Besilate and Hydrochlorthiazide in a combined dosage formulation. Present work describes simple, accurate, reproducible, rapid and economical methods for simultaneous estimation of Losartan Potassium, Amlodipine Besilate and Hydrochlorthiazide in tablet formulation.

MATERIALS AND METHODS

Reagents

Losartan Potassium, Amlodipine Besilate and Hydrochlorthiazide were obtained from Micro labs, Hosur, India. Acetonitrile and Methanol (HPLC grade, MERCK), water (Milli Q).Other reagents were of AR grade.

Instrumentation

The HPLC system consisted of a ShimadzuClass LC-10AT vp and Gelman science vacuum pumps connected with SPD-10A vp UV-Visible detector. The data acquisition was performed by Spinco Win chrome software.

Chromatographic conditions

The HPLC system consisted of ShimadzuClass LC-10AT vp and LC-20AD pumps connected with SPD-10A vp UV-Visible detector. The data acquisition was performed by Spincotech 1.7 software. Analysis was carried out at 238nm using a Phenomenex C18Reverse phase column of 250x 4.6 mm i.d., 5µm dimensions at ambient temperature. The mobile phase consisted of Phosphate buffer, Acetonitrile and Methanol in the ratio of 60: 20:20% v/v that was set at a flow rate of 1.0ml/min.

Preparation of standard stock solution

Stock solution was prepared by dissolving 250 mg Losartan potassium, 34.8 mg of Amlodipine besilate and 62.5 mg of hydrochlorthiazide in 100 mL was transferred to a 100ml volumetric flask, dissolved in 15ml of mobile phase, sonicated for 15 min and the volume was made up with mobile phase.

From the standard stock preparation 5ml of solution was taken in 50ml volumetric flask and further diluted with mobile phase. A volume of 20 µL of working standard was injected into column.

Preparation of mobile phase

Preparation of phosphate buffer:

6.8 gm of KH_2PO_4 was dissolved in 1000ml of water and adjusted to pH 6 with Potassium Hydroxide then mixed the above buffer.

Mobile phase

Phosphate buffer: Acetonitrile: methanol (60:20:20%v/v)

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Procedure for Sample Preparation

To determine the content of losartan potassium, amlodipine besilate and hydrochlorthiazide simultaneously in tablets (label claim: 50mg losartan potassium, 5mg of amlodipine besilate and 12.5mg of hydrochlorthiazide), twenty tablets were weighed, their average weight determined and were finely powdered. The correct amount of powder equivalent to 250mg of Losartan potassium was dissolved in mobile phase and sonicated for 30 min.

The excipients were separated by filtration. After filtration further dilutions are made with mobile phase. Then 5ml of clear solution was transferred into a 50 ml volumetric flask and diluted with mobile phase. And 20µl of this solution was injected for HPLC analysis.

Accuracy

To study accuracy of the method, recovery studies were carried out by addition of standard drug solution to sample at 3 different levels, 80%, 100% and 120%of the test concentration

Precision

Precision of the method was checked by system precision and repeatability (Intra day and Inter day studies). In system precision 6 replicates of mixed standard were used. Repeatability was done by using 3 replicate readings at 3 concentration levels. For Intraday variability trials are taken in a day and for Interday variability studies were done on 3 consecutive days.

Robustness

Robustness of the method was determined by small, deliberate changes in flow rate, mobile phase ratio, Wavelength of detection and pH of mobile phase. Flow rate was changed to 1 + 0.05 ml/min.

LOD and LOQ Determination

Limit of detection can be calculated by using following formula,

$$LOD = 3.3\ \sigma/S$$

Limit of quantitation can be calculated based on standard deviation of the response and the slope.

$$LOQ = 10\ \sigma/S$$

Where σ = Standard deviation of the response

S = Slope of the calibration curve

System Suitability Testing

System suitability testing is used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. Parameters such as theoretical plates, tailing factor, Resolution are determined and compared against the specifications and are presented in Table 1

RESULTS AND DISCUSSION

For HPLC method, chromatographic conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried for better separation of drugs . Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor, run time etc). The mobile phase was consisted of 0.02mM Phosphate buffer :methanol :acetonitrile with ratio of 60:20:20 % v/v at 1 mL/min flow rate was quite satisfactory. The optimum wavelength fixed for detection was 238 nm at which better detector response for drugs were obtained.the chromatogram of sample was shown in Figure1

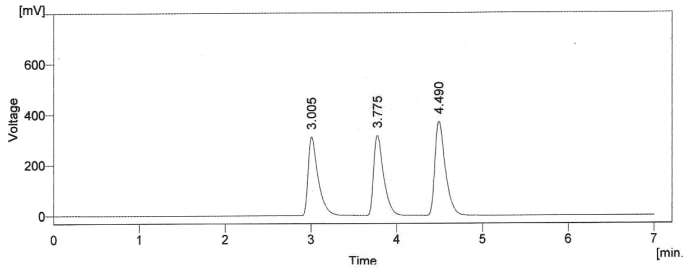

Fig.1: Chromatogram of working standard mixture of losartan potassium, Amlodipine besilate and Hydrochlorthiazide.

Method validation

System suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. The calibration was linear for losartan potassium at concentration range of 200.24-300.36µg/mL with the slope, intercept, correlation coefficient(r) were found to be 11.80, 24.63 and 0.9999 respectively and The calibration was linear for amlodipine besilate at concentration range of 27.84-41.76 µg/mL with the slope, intercept, correlation coefficient(r) were found to be 67.92, 11.132 and 0.9999 respectively , and The calibration was linear for hydrochlorthaizide at concentration range of 50.00-75.00 µg/mL with the slope, intercept, correlation coefficient(r) were found to be 38.60, 37.98, and 0.9999 respectively.

Sample to sample precision and accuracy were evaluated using, three samples of three different concentrations, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days, over a period of one week. These results show the accuracy and reproducibility of the assay. Thus, it was concluded that there was no significant difference on the assay, which was tested on an intra – day and inter – day basis. The % R.S.D. values was found to be less than 1% shows that proposed method provides acceptable intra – day and inter – day variation of hydrochlorthiazide, amlodipine besilate and losartan potassium with precision and accuracy. The mean recoveries were found in

the range of 98 – 100 %. The method was found to be simple, accurate, economical, rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.The validation parameters shown in table 2

Table 1: System suitability parameters

System Suitability Parameters	Hydrochlorthiazide	Amlodipine besilate	Losartan potassium
Resolution	3.733	3.265	
Tailing factor	1.6	1.6	1.545
Number of theoretical plate	3684	5053	6283
Retention time	3.008	3.775	4.492

Table 2: Validation Parameters of Losartan Potassium, Amlodipine Besilate And Hydrochlorthiazide

Parameters	Losartan potassium	Amlodipine besilate	Hydrochlorthiazide
Accuracy	% Recovery = 100.59	% Recovery = 99.74	% Recovery = 100.12
System precision	%RSD = 0.116	%RSD = 0.421	%RSD = 0.147
Method precision	%RSD = 0.376	%RSD = 0.613	%RSD = 0.182
Linearity	R^2 = 0.999	R^2 = 0.999	R^2 = 0.999
Range	200-300 µg/ml	27-41 µg/ml	50-75 µg/ml
LOD	1.522 µg/ml	0.051 µg/ml	0.139 µg/ml
LOQ	4.612 µg/ml	0.156 µg/ml	0.421 µg/ml

CONCLUSION

The method described enables the quantification of Losaratn potassium ,Amlodipine besilate and Hydrochlorthiazide in combined tablet dosage form. The validation data demonstrate good precision and accuracy, which prove the reliability of the proposed method. Hence this HPLC method can be used routinely for quantitative estimation of both components in solid oral dosage form.

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