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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF LOSARTAN POTASSIUMIN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Losartan potassium is the first of a unique class of oral antihypertensive agents referred to as angiotensin II receptor antagonistsis used to treat high blood pressure (hypertension). It's also used to lower the risk of stroke in some patients with heart disease. A simple, selective, precise, accurate and cost effective reverse phase HPLC method has been developed and validated for estimation of Losartan potassium in extended release tablet dosage form. In the chromatographic conditions, Hypersil ODS C18, 4.6×150 mm, 5 µm stationary phase with mobile phase consisting of Triethylamine solution (0.5%) pH 2.4 and acetonitrile 65:35 (v/v) was used at a flow rate of 1.0 mL/min. and column temperature was main at 30°C. Losartan potassium was detected at 225 nm. The chromatographic procedure separated Losartan potassium and potential interfering peaks in an analysis time of 5.0 min. with Losartan potassium eluting at about 2.7 min. The assay method was found linear in the concentration range of 0.05-100 µg/ml with a correlation coefficient of 0.9999. The percentage recovery of assay was found between 100.1 and 101.2. The developed method was validated with respect to specificity, linearity, accuracy, precision, sensitivity, robustness and solution stability as per ICH guidelines. The proposed method can be used for routine analysis of Losartan potassium formulations in quality control laboratories.

KEY WORDS

Losartan, HPLC, Validation, Dissolution, Extended Release

INTRODUCTION:

Losartan potassium, 2-butyl-4-chloro-1-[[2'(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1Himidazole-5-methanol monopotassium salt (Fig. 1), is the first member of a new class of nonpeptide angiotensin II receptor antagonist[1,2]. It reduces effectively hypertension by suppressing the effects of angiotensin II at its receptors, thereby blocking the renin-angiotensin system[3,4]. Losartan has been demonstrated to be superior to previous peptide receptor antagonists and angiotensin converting enzyme (ACE) inhibitors because of its enhanced specificity, selectivity, and tolerability[5]. Currently, losartan potassium is marketed alone or combined with hydrochlorothiazide.

Reverse Phase HPLC: In this chromatographic technique, the stationary phase is non-polar and the mobile phase is polar, non-polar compounds are retained for longer periods as they have more affinity towards the stationary phase. Hence, polar compounds travel faster and are eluted first.[3]

Steps involved in development of RP-HPLC method:

Selection of chromatographic method: The proper selection of methods depends upon the nature of the sample (ionic or ionisable or neutral molecule) its molecular weight and stability. The drug selected is polarand ionic hence reversed phase chromatography was used because of its simplicity and suitability.[4]

Selection of stationary phase: Matching the polarity of sample and stationary phase and using a mobile phase of different polarity achieve a successful separation.[5]

Selection of mobile phase: Reversed phase bonded packing, when used in conjunction with highly polar solvents; approach is ideal and is a universal system for liquid chromatography. Mobile phase may be either single liquid or combination of liquids, which are compatible with sample, column and instrument.[6]

Selection of suitable detector: Detector is the eye of HPLC system that measures the compounds after their separation on the column. There are basically two types of detectors- the bulk property detectors and solute property detectors. Detectors, in order of their popularity are UV, fluorescent, conductivity, polarimeter and refractive index detectors. UV detector is the first choice because of its convenience and applicability in case of most of the samples. The latest versions of equipment's are available with photo diode-array detectors (PAD or DAD).

Method optimization:

During the optimization stage, the initial sets of conditions that have evolved from the First stages of development are improved or maximized in terms of resolution and shape, plate Counts asymmetry, capacity, elution time, detection limits, limit of quantization, and overall ability to quantify the specific analyte of interest.

The literature reports many analytical methods for the quantitation of losartan in tablets using HPLC [7-11]. These methods employ mobile phases with buffer solutions and the separation of losartan degradants was not achieved with isocratic methods.In the current work we have made an attempt to develop simple, robust, cost effective and high throughput analytical method for the determination of Losartan potassium in tablet dosage form. The method uses UV detection with a run time of 5 min. The method has several advantages like simple mobile phase, low injection volume, less run time over the reported methods. The developed method was successfully validated as per ICH guidelines[12-14] and can be used in routine quality control analysis

MATERIALS AND METHODS

Drug profile of Losartan

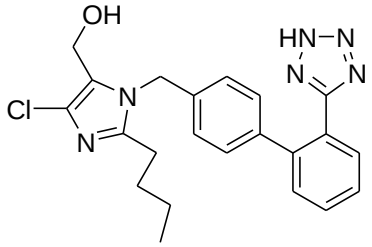

Fig.1.Structure of Losartan

IUPAC Name:2-butyl-4-chloro-1-[[2'(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1Himidazole-5-methanol monopotassium salt

Chemical formula: $C_{22}H_{22}ClKN_6O$

Molecular weight: 461.007

Description: White to pinkish crystals or purplish-tan powder.

Solubility: Soluble in water, soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide.

Category: Angiotensin II receptor antagonist

λ_{max}: 225 nm

Drugs Used:

Table: 1. List of Drugs Used

S. No.	Drugs	Manufacturer
1.	Losartan potassium	Hetero Drugs Ltd
2.	New formulation ER 50 mg tablets	Unichem Ltd

Reagents Used:

Table: 2. List of Reagents used

S. No.	Chemicals	Manufacturer name	Grade
1	Water	Merck	HPLC
2	Methanol	Merck	HPLC
3	Acetonitrile	Merck	HPLC
4	Triethylamine	Merck	HPLC

Equipment and Apparatus Used:

Table: 3. Equipment and Apparatus Used

S.No.	Instrument Name	Model Number	Software	Manufactures Name
1	HPLC	Alliance UV-Visible detector-2487	Empower	Waters
2	U.V Double beam spectrophotometer	SL 210	-	ELICO
3	Digital weighing balance (Sensitivity 5 mg)	BL-200H	-	SHIMADZU
4	PH-meter	LI-120	-	ELICO
5	Sonicator	3305013	-	SISCO

Preparation of mobile phase:

A combination of Mobile phase containing Triethylamine solution (0.5%) pH 2.4 and acetonitrile 65:35 (v/v) was mixed and degassed in ultrasonic water for 5 minutes finally filtered through 0.45µm membrane filter. This prepared solution was used as mobile phase.

Diluents:

Water and acetonitrile in theratio of 50:50 (v/v) was used as diluent

Preparation of standard solution: (0.05mg/ml)

Accuratelyweighed 25 mg of Losartan potassium working standard into a 50 mL volumetricflask, added25 mL of diluent, mixed to dissolve and made up the volume with diluent.From the standard stock solution, standard solution was prepared to contain, 50 µg/ml of Losartan potassium.

Preparation of sample solution: (0.05mg/ml)

20 tablets were crushed to powder, weighed and transferred the tablet powder equivalent to 50 mg of Losartan potassiuminto 250 mL volumetric flask added70 mL of diluent, sonicated for 10 minutes and diluted to volume with diluent. Filtered the solution through 0.45µnylon filter. An aliquot portion of the filtrate was further diluted to get final concentration of50µg/ml with diluent.

Wavelength selection:

About 0.25mg/mL of Losartan potassiumsolution was accurately prepared by dissolving the active in water. The Cilostazol solution scanned in the 200-400 nm UV regions. The wavelength maximum (λmax) was observed at 250 nm and this

wavelength was adopted for absorbance measurement.

Optimized chromatographic conditions:

Column: Hypersil ODS C18, 4.6×150 mm, 5 µm
Column temperature: 30°C.
Wave length: 225nm
Mobile phase ratio: Triethylamine solution (0.5%) pH 2.4 and acetonitrile 65:35
(v/v)
Flow rate: 1.0 min/ml
Injection volume: 20µl
Run time : 5minutes

Validation of developed RP-HPLC method:

As per the International conference on harmonization (ICH) guidelines the method validation parameters such as linearity, precision, accuracy, system suitability, limit of detection and limit of quantitationwere optimized.

Assay

Sample and standard was injected into the chromatographic system and measured the area for perindopril and calculated the % assay by using the formulae.

Calculation:

$$Assay\ \% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

Where :
Avg.wt = average weight of tablets
P = percentage purity of working standard
LC = label claim of Losartan potassium mg/ml

RESULTS AND DISCUSSION:

Optimized method:

It was performed on Hypersil ODS C18, 4.6×150 mm, 5 µm with a mobile phase composition of Triethylamine solution (0.5%) pH 2.4 and acetonitrile 65:35 (v/v) at a flow rate of 1.0 min/ml. 20µl of sample was injected and the run time was 5 minutes.

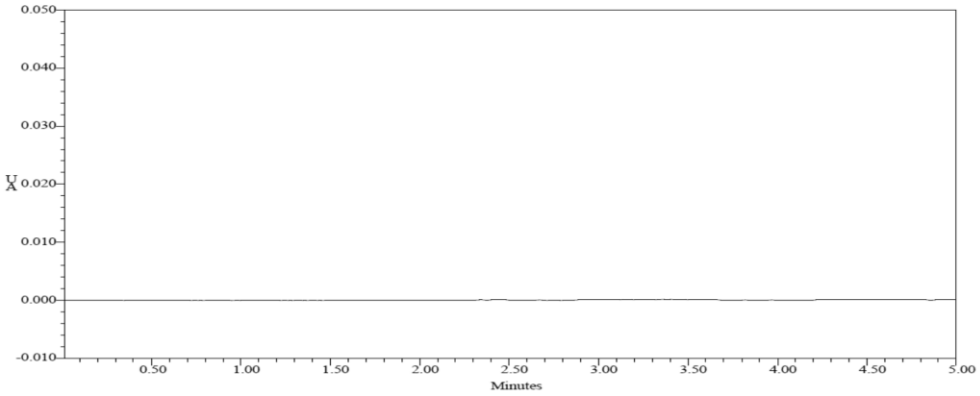

Fig. 2: Chromatogram showing blank preparation (mobile phase)

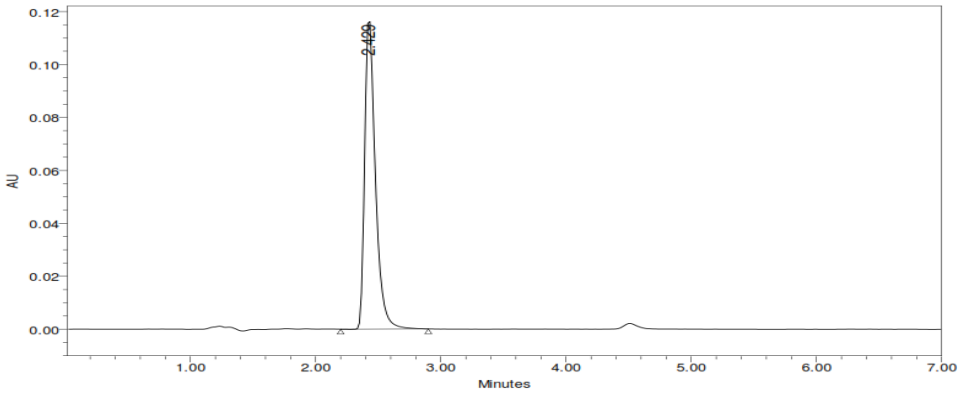

Fig. 3: Chromatogram of Losartan potassiumstandard peak

Linearity:

25, 37.5, 50, 60, 75µg/ml was injected into the chromatographic system and peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and Y-axis peak area) and the correlation coefficient was calculated.

Acceptance criteria:

Correlation coefficient should be not less than 0.999.

Table 4: Showing the results for the linearity

Conc.(µg/ml)	RT	Area
25	2.527	1102148
37.5	2.523	1650299
50	2.524	2204968
60	2.522	2753639
75	2.523	3302058
Co efficient of correlation(R^2)		0.998

Precision:

The standard solution (0.05 mg/ml) was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria:

The %RSD for the area of five standard injections results should not be more than 2.

Table 5: Showing the results for Precision

S. No	Conc. (µg/ml)	Rt	Area
1	50	2.522	2301563
2	50	2.553	2301492
3	50	2.546	2301925
4	50	2.535	2301636
5	50	2.545	2301968
Mean			2301717
Std. Dev			216.31
%Rsd			0.01

Accuracy:

The standard solution of concentration 25, 50 and 75 ppm were injected into chromatographic system. Calculate the amount found and amount added for Cilostazolcalculated the individual % recovery and mean % recovery values.

Acceptance criteria:

The % recovery for each level should be between 98.0 to 102.0%.

Table 6: Showing Accuracy results for Losartan potassium

S. No	Conc(µg/ml)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean% recovery
1	25	1112116	2.5	2.502	100.1%	100.1%
2	50	2201546	5	5.06	101.2%	
3	75	3209069	7.5	7.506	100.1%	

System suitability:

The standard I solution was injected one time and standard II solution was injected 5 times.

Table 7: Showing System Suitability results for Losartan potassium

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	7679	1.0
2	1.0	7865	1.0
3	1.2	7969	1.1

Limit of detection (LOD)

From the above preparation 1ml of solution is transferred to 10ml of volumetric flask and the volume made with the diluents.

Table.No.8. Showing results for Limit of Detection

Drug Name	y-Intercept	Slope(s)	LOD(µg/ml)
Mesalamine	1250	$5.49*10^3$	2.79

Limit of quantitation (LOQ)

From the above preparation 0.5ml of solution is transferred to 10ml of volumetric flask and the volume made with the diluent.

Table.No.9. Showing results for Limit of Quantitation

Drug Name	y-Intercept	Slope(s)	LOQ(µg/ml)
Losartan potassium	1250	$5.49*10^3$	8.33

Assay:

The developed and validated method was applied to the determination of Mesalaminein marketed tablets containing 100 mg of drug per tablet. Three injections of sample were injected into chromatographic system. Assay % was calculated by using the formula mentioned above and it was found to be 99.8%.

Table 10: Showing the results of assay

S. No	Name	Rt	Area
1	Losartan potassium	2.548	2301243
2	Losartan potassium	2.581	2301792
3	Losartan potassium	2.024	2309596

Conclusion:

A simple, rapid, accurate and precise RP-HPLC method was developed for the determination of Losartan potassiumin pure form and in tablets. The analytical conditions and solvent system developed provided a good separation forLosartan potassiumwithin a short analysis time. The method was validated and demonstrated a wide linear dynamic range, a good precision and accuracy. Thus, the method can be proposed for routine analysis laboratories and for quality control.

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