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Simple and stability indicating RP-HPLC assay method development and validation lisinopril dihydrate by RP-HPLC in bulk and dosage form

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KEYWORDS

Lisinopril dihydrate (LSD), Sodium Lauryl sulphate (SDS), RP-HPLC, Method development and validation

A B S T R A C T

A new simple, accurate, precise, sensitive and validated by RP-HPLC was developed for the estimation of Lisinopril dihydrate in bulk and pharmaceutical dosage form. The Chromatographic conditions used for the separation was X BRIDGE C18 (50 mm x4.6 mm, 3.5µm) and the mobile phase comprised of Acetonitrile and SDS Buffer (30:70 v/v). The flow rate was 0.5 ml/ 1minute with the detection at 210 nm. The Assay method was validated as per ICH guidelines. The retention time was found to be 4.72 minutes. The linearity was found to be in the range of 0.1 – 0.6 mg/ml (25% to 150%) with correlation coefficient(r) 0.996. The proposed method is accurate with 99.9% - 99.98% recovery for Lisinopril dihydrate and precise. %RSD of repeatability, intraday and inter day variations were 0.174 - 0.63. The method can be successfully applied to pharmaceutical formulation.

Introduction

Lisinopril dihydrate (LSD) is chemically(S) - 1-[N2-(1- carboxy-3-phenylpropyl)-L-lysyl]-L-prolinedihydrate. It is also used as Antihypertensive agent. [1] Lisinopril is a potent, competitive inhibitor of angiotensin-converting enzyme (ACE), the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the rennin angiotensin-aldosterone system (RAAS). [2-4] Lisinopril may be used to treat hypertension and symptomatic congestive heart failure, to improve survival in certain individuals following myocardial infarction and to prevent progression of renal disease in hypertensive patients with diabetes mellitus and microalbuminuria or overt nephropathy. [5-6]

Lisinopril Dihydrate is available in strength of 5mg tablet dosage form. The strength of

tablet is analyzed with the developed method.

Lisinopril Dihydrate has some published methods for estimation of assay and impurity profile by HPLC and UV/visible spectroscopy techniques. The objective of the research is to develop a simple RP-HPLC Assay method. Method validation has performed as per the ICH and regulatory guidelines and review articles were revealed for method development and validation.

Materials and Methods

Reagents and materials

The reference sample of Lisinopril Dihydrate was supplied as a gift sample from Hetro Labs Ltd. Hyderabad, Telagana. The commercially available Lisinopril dihydrate (LISTRIL-5) solid dosage forms were procured from the local market. Triple distilled Milli-Q-water was used throughout this research. HPLC grade Acetonitrile, Milli-Q-water, analytical grade SDS, H_3PO_4 and TEA was used in this method.

Chromatographic parameters

The chromatography was performed on a LC 10 AT vp HPLC instrument (Shimadzu Corporation, Japan) equipped with SPD-10A vp detector, SCL-HT A auto sampler and CTO-10A vp Column oven. The data was monitored with LC solutions software. X BRIDGE C18 (50 mm x4.6 mm, 3.5µ, Agilent technologies, USA) was used as stationary phase. Sartorius BT 224s analytical balance was used for this research experiments. The flow rate was set at 0.5 µl/min. The detection was monitored at 210 nm and the retention time was 4.72 minutes. An injection volume of 10 µL was used for the analysis. The column temperature was maintained at 60°c.

Selection of mobile phase

Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different mobile phases. From the various mobile phases tried, the following compositions were given good response to the Drug solution.

200 mg of SDS dissolved in 400 ml of mill-Q-water mixed thoroughly, and to this add 8ml of H_3PO_4 and 8 ml of TEA were added then prepared to 500 ml volume.

Mobile phase was prepared by mixing SDS buffer and Acetonitrile in the ratio of (30:70 v/v. The mixture was filtered and degassed through 0.45 µm membrane filter paper.

Preparation of diluent

Diluent was prepared with acetonitrile and SDS buffer with 30: 70 (v/v) ratio and degassed with 0.45µ filter.

Preparation of standard stock solutions

25 mg of Lisinopril dihydrate standard was accurately weighed and transferred into a 25 ml of volumetric flask and was initially dissolved in 10 ml of diluent. The solution is then made up to a volume so as to obtain a stock solution of 1 mg/1 ml. From the stock suitable dilutions were prepared.

Preparation of calibration curve

Aliquots of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml standard stock solution was transferred to the 10 ml of volumetric flasks and made up to the mark with mobile phase to get concentration of 10-60µg/ml.. The fixed standard solution was prepared by transferring 3 ml of Lisinopril Dihydrate to

10 ml of volumetric flask and made up to the mark with mobile phase to get 30 µg/ml of Lisinopril dihydrate .

Sample preparation

Two tablets of LISTRIL-5 mg were taken and recorded the weight. Ground the tablets in a Agate mortar and weighed a quantity equivalent to 10mg (174mg) of Lisinopril Dihydrate. Transferred to 10 ml standard flask and added 5ml of diluent. The flask was shaken 15 min and diluted to the mark with diluents. The solution was then filtered through 0.45 micron membrane filter. Transferred 3ml to 10 ml volumetric flask dilute and made up to the mark with mobile phase.

Results and Discussions

Method development

To develop simple and stability indicating RP-HPLC method for Lisinopril dihydrate determination, several research experiments were performed with different salt and acid buffers and mobile phase compositions. Finally, satisfactory separation with high peak symmetry were obtained with X BRIDGE C18 (50 mm x4.6 mm, 3.5µm Agilent technologies, USA) and the mobile phase comprised of Acetonitrile and SDS Buffer (30:70 v/v) at a flow rate of 0.5 ml/min. Quantification was achieved at 210 nm based on peak area. The retention time was found to be 4.72min. The optimized method was validated as per ICH guidelines. System suitability, specificity, linearity, accuracy, robustness and ruggedness were performed.

Method validation

The optimized Chromatographic method has high accuracy, linearity and all method validation parameters were performed and reported below.

System suitability test

10µL of the standard solution (0.3 mg/ml) was injected under optimized chromatographic conditions to evaluate the suitability of system. The values of system suitability parameters were shown in Table 2.

Specificity

Specificity of the RP-HPLC method was demonstrated by the separation of the analytes from other potential components such as impurities and excipients. A volume of 10 µl of working placebo sample solution was injected and the chromatogram was recorded. No peaks were found at retention time of 4.72min. Hence, the proposed method was specific for Lisinopril dihydrate.

Linearity

The linearity of calibration curve in pure solution was carried over the concentration range of 0.1-0.6 mg/ml through proposed RP-HPLC method. The data was represented in Table 3. The Correlation Coefficient is 0.999558 indicates that the method is Linear.

Precision

The precision of the method was determined by injecting 0.3 mg/ml concentration in replicate (5 times).

Repeatability

The Repeatability of the proposed method was ascertained by injecting five replicates of fixed concentration within the Beer's

range and finding out the peak area by the proposed method. The method precision was carried out by intraday and inters day measurement. From this peak area % RSD was calculated. (Table 4) The calculated %RSD observed is well below 0.17 indicates that the method is Precise.

Accuracy

For the accuracy of proposed method, recovery studies were performed by standard addition method at five different levels (25%, 50%, 75 %, 125% and 150% of final concentration). A known amount of standard pure drug was added to pre-analyzed tablet powder and the sample was then analyzed by proposed method. Results of recovery studies were found to be satisfactory and reported in Table 5a and 5b.

Stability of the analytical solutions

The stability of the drug is determined by placing the sample solution for the short term stability by keeping at room temperature up to 24 hours and then comparing the obtained peak area with that of the similarly prepared fresh sample. Further, auto-sampler stability for up to 24 hours was studied and established. The result indicates that the sample solution is stable upto 24h

Stress degradation

The following conditions were observed for study of the stability of the drug-

a. The drug is mixed with 10 ml of 0.05 N HCl and kept for 24 hours
b. The drug is mixed with 10 ml of 0.051 N HCl and kept for 24 hours
c. The drug is mixed with 10 ml of 0.1 NaOH and kept for 24 hours
d. The drug is kept in oven at 80^0 C for 24 hours
e. The drug is placed in sunlight (UV) for a day.

The stress studies involving acid 0.05N, 0.1N HCl, light (UV) and heat (80^0C) revealed that Lisinopril dihydrate was stable under the stress conditions. For all stress conditions studied, the drug content was within 97.5 – 99.1 % indicating the stability and specificity of the analytical method to differentiate the degradation peaks. The peak purity of Lisinopril dihydrate main peak in all stress conditions met the requirement. It indicates that the method is specific for Lisinopril dihydrate .

Robustness

Robustness was studied by deliberately changing the Flow rate and Temperature of the column. Analyzed the standard solution was changing the flow rate about ± 0.1 µl to the original flow rate 0.5 µl and also recorded the analysis data for changing the column oven temperature about ± 2^0C to the original 60^0C temarature. Method precision verified with different Flow rates and Temperatures. The % RSD for 0.4µL, 0.6µL and 58^0C, 62^0C is within the limits.

Conclusion

A rapid and reliable isocratic RP-HPLC-UV method for the determination of Lisinopril Dihydrates has been developed and validated. This chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method. It is highly simple, accurate, precise, sensitive, validated and analytical procedure and its retention time 4.72 min allows the analysis of large number of samples in a short period of time. So this can be used for routine analysis.

Table.1 Optimized chromatographic conditions

Mobile phase	Acetonitrile and SDS Buffer (30:70 v/v)
Stationary phase	X BRIDGE C18 (50 mm x4.6 mm, 3.5µm)
Wavelength	210nm
Run time	15 min
Flow rate	0.5 ml/min
Injection volume	10µl
Temperature	60^0C
Mode of operation	Isocratic elution
PH	4.6

Table.2 System suitability test parameters

System suitability parameters	Result
Retention time	4.72
Area	11664562(0.3mg/ml)
Theoretical plate number	1363.4
Tailing factor	1.369

Table.3 Linearity data

Linearity level	Concentration (mg/ml)	Peak area
1	0.1	3913743
2	0.2	7810285
3	0.3	11664562
4	0.4	15274318
5	0.5	18729453
6	0.6	22122698
Slope		364034.4
Intercept		511306.3
Regression equation		Y=364034.4 x ±511306.3
Correlation Coefficient®		0.999558
Coefficient of determination(r^2)		0.9992

Table.4 Precision data

Precision	
Repeatability (%RSD,n=5)	0.17
Intraday Precision(%RSD,n=5)	0.17-0.45
Interday Precision(%RSD,n=5)	0.45-0.63

Table.5a Accuracy results

Drug name	Levels	Amount added(mg/ml)	Lisinopril dihydrate content(mg)	Percent recovery	Average of percentage recovery
Lisinopril dihydrate	25%	0.1	25.62	99.9	99.96
	50%	0.2	51.13	99.96	
	75%	0.3	76.36	99.98	
	125%	0.5	122.6	99.98	
	150%	0.6	144.83	99.98	

Table.5b Accuracy results for tab

Brand name	Drug name	Amount labeled	Amount found	%Recovery	Average area	Standard deviation	%RSD
LISTRIL-5 Torrent pharm.Ltdsikkim	Lisinopril dihydrate	10mg (2 Tab)	10.78	107.8	12580347	13104.1	0.0104

Figure.1 Chemical structure of Lisinopril dihydrate

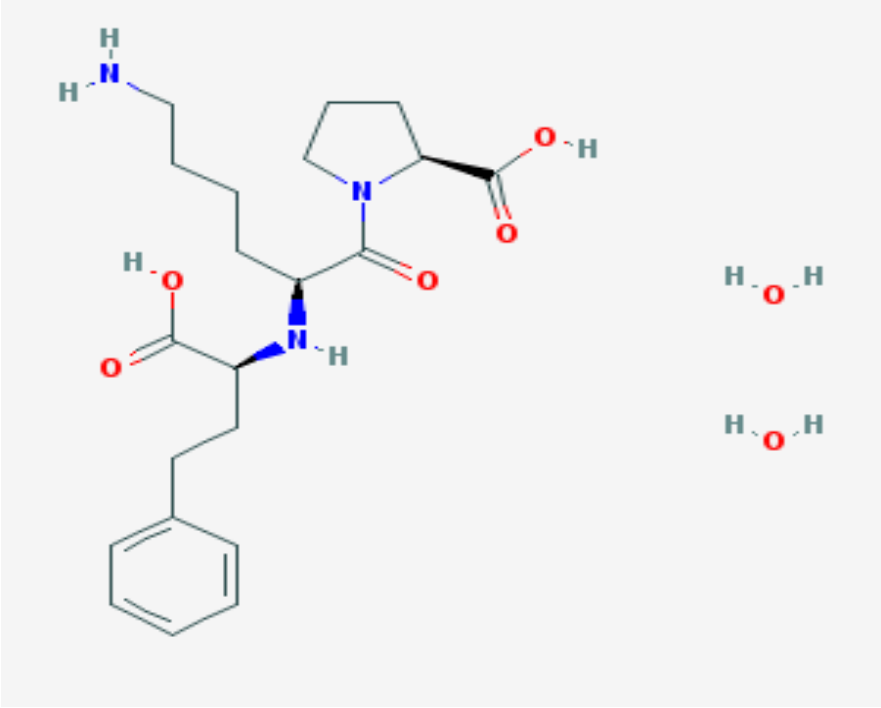

Figure.2 Linearity graph

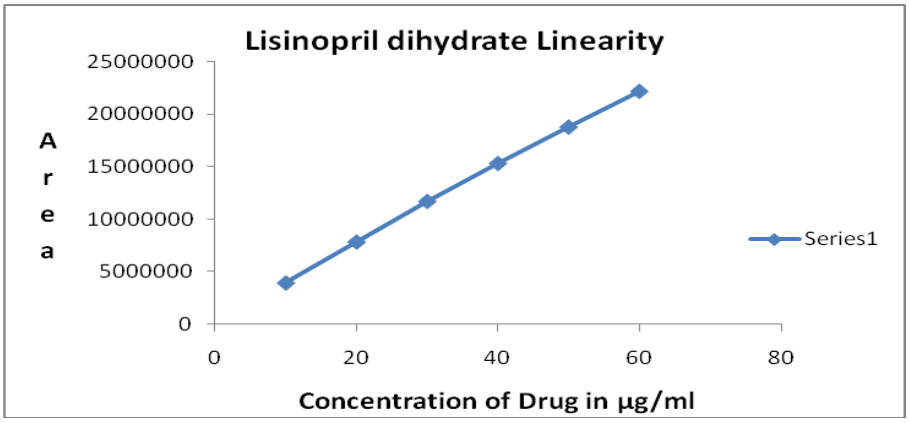

Figure.3 Lisinopril Dihydrate Standard precision chromatogram overlay

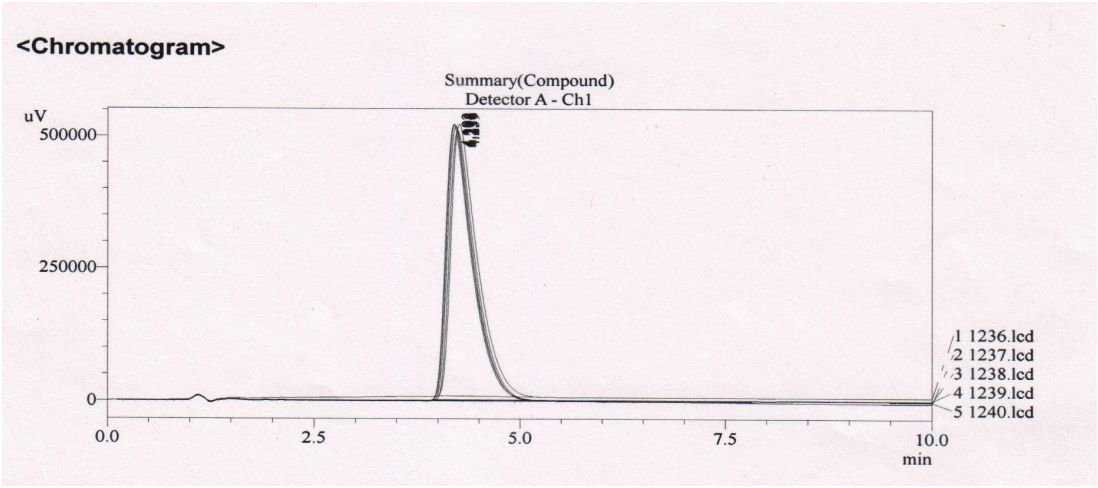

Figure.3 Lisinopril Dihydrate Standard and Tablet chromatogram overlay

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