Stability Indicating HPLC Method using Core Shell Stationary Phase for the Determination of Related Substances in Levocetirizine Dihydrochloride Oral Solution

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ABSTRACT

Introduction: Levocetirizine (LCZ) is a new generation antihistamine drug used for the allergic symptoms resulting from various diseases. The present research work focuses on the development of a simple and precise HPLC method for the effective separation and quantitative determination of LCZ and its impurities. **Objectives:** Eight potential related impurities of LCZ were separated and identified in the bulk drug as well as oral solution dosage form. **Results:** The separation was achieved on a core shell stationary phase Kinetex bipheyl (250, 4.6 × 5 μm) column with mobile phase of sodium perchlorate in water and acetonitrile in a gradient elution. The results were monitored and analytes were quantified at 230 nm. **Conclusion:** The proposed method was stability indicating and was validated as per ICH guidelines for specificity, linearity, precision, accuracy and robustness. The proposed method finds its application in the routine analysis of LCZ in bulk drug and various dosage forms.

Key words: HPLC, Core shell, Related Substances, Levocetirizine dihydrochloride.

INTRODUCTION

Levocetirizine (LCZ) is a third generation piperizine derivative indicated to be used as an effective antihistaminic (H1 blocker).[1] LCZ is the active enantiomer of cetirizine. It is commonly prescribed to relieve from allergic symptoms like watery eyes, running nose, sneezing, hives, and itching. LCZ is commonly taken as an oral pill.[2-6] The purity of the pharmaceuticals is of utmost concern and the presence of various impurities in the bulk drug and finished pharmaceuticals greatly affect the compliance of the product.[7] Thus it becomes important to provide good and efficient analytical methods which would be able to determine the analyte of interest in a simple, precise and accurate manner. The use of core shell technology stationary phases in the arena of separation

science is certainly gaining more importance due to its good chromatographic properties like good peak shape and retention properties.[8-10] The lifetime of these columns are better when compared to that of conventionally used reverse phase columns. Thus developing analytical methods which involve using the core shell columns provides definitive advantages over traditionally used columns in terms of reproducibility and repeatability of the study.[11-13]

The literature showed various analytical methods reported for the determination of Levocetirizine using HPLC in bulk and dosage forms, in plasma and other biological matrices. Almost all the reported methods indicated the usage of conventional stationary phases.[14-19] This motivated the authors to

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develop simple, precise, accurate and robust HPLC method using the core shell technology stationary phase for the determination of LCZ in bulk and formulations.

EXPERIMENTAL

Materials

LCZ was obtained as a gratis sample from Hetero Labs Pvt. Ltd., Hyderabad. HPLC grade acetonitrile and ammonium acetate were purchased from Merck (Darmstadt, Germany). AR grade glacial acetic acid was procured from SD Fine-Chem. Ltd. (Mumbai, India). High purity water was obtained from Millipore Milli - Q plus system (Milford, MA, USA). Water and acetonitrile (50:50 v/v) was used as the diluent and all the final solutions were made in the diluent.

Instrumentation

The liquid chromatographic analysis was performed on an Waters 2695 series HPLC (Waters Corporation) equipped with quaternary solvent manager, a degasser, an ultraviolet detector, an auto sampler and a column compartment with temperature regulation facility. The data acquisition was carried out using the Empower software (version 3)

Chromatographic conditions

The chromatographic separations were carried out on Kinetex bipheyl (250mm x 4.6 mm x 5μm). The mobile phase composed of buffer solution (6.0 gm of sodium perchlorate monohydrate in 1000 mL water along with 2 mL of perchloric acid) of pH 2.0 and acetonitrile in gradient elution. The gradient program set to achieve the separation was 0 /20, 70/25, 80/30, 90/30, 100/40, 110/40, 115/70, 125/70, 130/20, 140/20. The flow rate was 1.5 mL min^{-1} and the detection was achieved at 230 nm. The injection volume was 50 μL with column oven maintained at 45ºC.

Preparation of LCZ standard solution

The drug substance LCZ was accurately weighed (40 mg) and transferred into a 100 mL flask. About 60 mL of diluent was added and sonicated to dissolve the drug, then made up to the volume with diluent. 5 mL of the above solution was diluted to 100 mL with diluent to result the secondary stock solution. From the secondary stock solution a working standard solution was prepared (1:40 v/v) with the diluent. The stock solution and also the working standards were stored at 10°C and were found to be stable for several days.

Preparation of Sample

The oral solution of LCZ equivalent to 10 mg of LCZ was weighed into 100 mL flask; 40mL diluent was added to dissolve the contents and final volume made up with the diluent to 100 mL. The solution was sonicated for 30 min with intermediate shaking and then the was filtered through 0.45μm membrane filter.

Method Validation

The developed analytical method was validated according to the ICH Q2 (R1) guidelines.[20] The various parameters which were evaluated under the validation process

Precision

The precision of an analytical procedure indicates the closeness of agreement between a set of measurements obtained from multiple sampling of the same sample under the prescribed conditions. For system precision and method precision standard solution and sample solutions (unspiked and spiked) were prepared as per test method and injected into the chromatographic system respectively.

Accuracy

It shows the closeness of the true value which is accepted or standard and the value which is obtained after the experimentation. In accuracy study, LCZ and its impurities were spiked to the placebo and sample solutions respectively at a concentration ranging from LOQ to 150%. All the samples were injected in triplicate at each level.

Linearity and range

The linearity of an analytical method is the ability of the method to respond in direct proportion to that of concentration. The specific concentration levels within which the method shows a direct relation between the response and concentration is known as the linearity. The linearity was established from LOQ to 150% of the specification level of LCZ and the impurities.

Robustness

It shows the ruggedness of the method when deliberate but small changes are made to the parameters of the analytical method. This parameter builds in confidence into the method indicating the extent to which the method is capable of sustaining small variations while transfer of the method. To demonstrate the robustness of the proposed method in the present study, deliberate changes in the flow rate and column temperature were made. The observations were made by evaluating the

system suitability parameters of the standard and % RSD for the sample solutions.

Limit of detection (LOD) and limit of quantification (LOQ)

The determination of limit of detection and quantification of the drug and its impurities indicate the sensitivity of the method. It provides an estimate about the minimum levels from where the analytes can be invariably determined using the analytical method. LOD is the concentration where the signal to noise ratio is 3:1 and for LOQ it is 10:1.

RESULTS AND DISCUSSION

Optimization of HPLC conditions

The development of a HPLC method for the determination of LCZ and its related substances was aimed at proposing a stability indicating method that can quantify LCZ in the presence of its excipients with good precision and accuracy (Figure 1). The first step was to find an appropriate wavelength where LCZ and all its impurities can be effectively determined without any compromise (which is commonly observed for compounds with different absorption maximum). A suitable wavelength was found experimentally after measuring spectra of the components to be separated and subsequently testing the detector response of analytes at 230 nm. LCZ and its related substances showed different affinities and thereby various retentions for chromatographic stationary phases attributed to their differences in molecular structures (Figure 2).

The selection of suitable column for the separation of LCZ and its 9 impurities was critical. The approach was based on column screening studies where a number of columns with different chemistries were tried. Finally, Kinetex biphenyl column produced the best separation with optimum resolution among the various critical pairs. The organic component of the method was chosen with respect to obtain good resolution and low back pressure. The column temperature was maintained at 45ºC. The retention of the analytes on the stationary phase is dependent on the pH of the aqueous component. This determines the analytes to be either in ionized or neutral form thereby exerting an effect on their retention behaviour owing to interactions based the polarities of stationary phase and the molecules.

The aim of the present study is to develop a reversed-phase high-performance liquid chromatography procedure for the determination of LCZ in the oral solution dosage forms. LCZ is relatively non-polar compound as indicated by partition coefficient (log p = 4.94

Figure 1: Chromatogram of a) Blank, b) Placebo, c) Standard, d) Unspiked sample, e) Spiked sample.
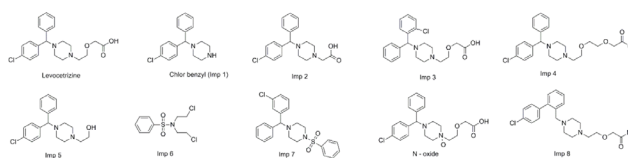

Figure 2: Structures of LCZ and its impurities.

and 6.03) [15], and is well retained on traditional C18 bonded phases. The initial trials were carried out with aqueous buffer solutions of pH 3.0, 4.0, 5.0, 5.5 and 6.0 with organic phase being methanol or acetonitrile. The method was optimized in keeping view of adequate separation of the degradation products from the main peak.

The developed chromatographic method was capable of determining LCZ in its oral solution dosage forms.

Method validation

The developed method was validated to establish the specificity, precision, linearity, accuracy and robustness according to ICH guidelines.[16]

In the present study where the separation of 9 impurities was carried only two impurities (Imp 1 and N-Oxide impurity) were quantified. Since the other related impurities are process related they need not be quantified and were included to confirm their relative retention time (Shown in Table 1).

Specificity

The specificity of the method was performed by ensuring the separation of the drug from all the interferences Figure 2. The specificity of the method was established by evaluating the peak purity of LCZ and all its impurities. The results showed LCZ to be pure and all the degradation products were separated and no other peaks were interfering with it (Table 1).

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of the developed method were determined by injecting sample solutions of LCZ and

Table 1: System Suitability Parameters.

Identification and retention time confirmation			SST parameters for LCZ			
Compound	**Retention Time**	**Spiked samples**	**S.No**	**Peak area**	**Theoretical plates**	**Tailing factor**
	Individual identification solutions					
LCZ	72.891	72.172	1	30869	51985	0.98
Imp 1	46.449	46.441	2	33087	49387	0.89
Imp2*	55.477	55.469	3	32644	59171	0.92
Imp5*	59.262	59.234	4	32622	51228	0.96
Imp8*	61.433	61.327	5	34275	44282	0.92
Imp3*	69.799	69.759	6	33565	48514	0.86
Imp4*	76.435	76.483	Mean	32844	50761	0.92
Imp6*	78.406	78.496	SD	1150.35	-	-
N-oxide Imp	84.996	84.875	%RSD	3.50	-	-
Imp7*	103.903	103.861				

*As these are process related impurities, given for relative retention time confirmation not for quantification.

Table 2: Linearity for LCZ, Imp 1 and N-Oxide Imp.

LCZ					
Level	**Concentration (in ppm)**	**Mean Peak Area**	**% RSD**	**Statistical Analysis**	
LOQ	0.048	3418	6.35	Correlation coefficient (r)	0.99926
Level-1	0.250	15720	-	r 2	0.99853
Level-2	0.376	24987	-	Slope	64730.04
Level-3	0.501	33570	-	y-intercept	301.82
Level-4	0.626	41118	-	%y-intercept	0.90
Level-5	0.751	48189	2.20	-	-
Imp 1					
Level	**Concentration (in ppm)**	**Mean Peak Area**	**% RSD**	**Statistical Analysis**	
LOQ	0.041	4380	6.77	Correlation coefficient (r)	0.99984
Level-1	0.249	28820	-	r 2	0.99968
Level-2	0.374	41353	-	Slope	112354.14
Level-3	0.499	56366	-	y-intercept	33.77
Level-4	0.623	69834	-	%y-intercept	0.06
Level-5	0.748	84155	1.36	-	-
N-Oxide Imp					
Level	**Concentration (in ppm)**	**Mean Peak Area**	**% RSD**	**Statistical Analysis**	
LOQ	0.050	3235	2.58	Correlation coefficient (r)	0.99934
Level-1	0.250	14286	-	r 2	0.99868
Level-2	0.375	22602	-	Slope	61961.38
Level-3	0.500	29795	-	y-intercept	-570.09
Level-4	0.625	38155	-	%y-intercept	-1.91
Level-5	0.750	46508	2.17	-	-

all its impurities individually by progressively decreasing the concentration of the analyte (Table 3).

Linearity

The linearity of the method was checked by injecting six concentration levels of LCZ, Imp 1 and N-Oxide impurity ranging from LOQ to 150% of specification level. Each solution was injected in triplicate and the mean was taken for calculation. Calibration equation was obtained from linear regression analysis and the correlation coefficient was found to be 0.99961which shows that the method is linear. The regression equation for the

calibration curve was found to be y = 32224.51x + 46741 (Table 2).

Accuracy

The accuracy of the method was evaluated in triplicate at three concentration levels, i.e. 50%, 100% and 150% levels by spiking the drug LCZ and all the impurities in tablet placebo. The percentage of recovery of the drug was calculated at each level. The percentage recovery of the LCZ and its impurities ranged from 98.9% to 100.7% indicating the accuracy of the method (Table 5).

Precision

The precision studies are performed to evaluate the repeatability of the method. The repeatability of the proposed method was demonstrated by analyzing six separate samples solutions of LCZ spiked with its 8

impurities (0.1 % level with respect to LCZ concentration). The %RSD for the peak areas of different injections were calculated and were found to be less than 2 showing the method to be highly precise. The results are shown in Table 4.

Robustness

The experimental conditions were deliberately changed in order to determine the robustness of the developed method and percentage assay and tailing factor of the main peak was evaluated. Observation of the results for deliberately changed chromatographic conditions (flow rate, and column temperature) revealed percentage assay and also the tailing factor were within the limits, illustrating the robustness of the method.

Table 3: LOD and LOQ data of LCZ.			
	Component	Concentration (in %)	S/N ratio
	Imp 1	0.013	5
LOD	LCZ	0.016	4
	N-Oxide Imp	0.017	5
	Imp 1	0.041	13
LOQ	LCZ	0.048	11
	N-Oxide Imp	0.050	15

Table 4: Precision data.			
Validation Parameter	Results		
System Precision	Minimum theoretical plates	44481	37116
	Maximum tailing factor	0.99	1.02
	%RSD	3.04	3.98
	Impurities	% RSD	
		Unspiked Samples	Spiked samples
Method Precision	Imp 1	NA	2.12
	N-Oxide Imp	NA	3.43
	Maximum individual unspecified impurity	NA	NA
	Total Impurities	NA	2.67
Intermediate Precision	Imp 1	1.20	3.07
	N-Oxide Imp	1.65	2.82
	Maximum individual unspecified impurity	NA	NA
	Total Impurities	0.56	2.62

Table 5: Accuracy.					
	Component	% Recovery			
		LOQ	50% level	100% level	150% level
Accuracy	LCZ	103.1	100.0	101.0	101.9
	Imp 1	101.9	102.1	102.1	105.7
	N-Oxide Imp	105.5	96.5	103.1	106.6

CONCLUSION

A simple, precise and accurate HPLC method was developed to separate and quantify LCZ and its related impurities. The developed method was validated according to ICH guidelines and demonstrated to be specific, linear, precise, accurate and robust. The specificity results indicated the stability indicating power of the proposed chromatographic method. The method can be successfully employed in regular analysis and quality control laboratories.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

LCZ: Levocetrizine dihydrochloride; **HPLC:** High Performance Liquid Chromatography; **ICH:** International Conference on Harmonization; **%RSD:** Percent relative standard deviation.

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PICTORIAL ABSTRACT
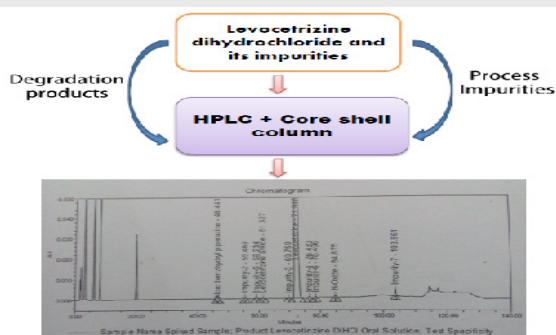
SUMMARY

- The research work focuses on the utility of monolithic columns in efficient separation of LCZ and its impurities. It also emphasizes on the important aspect of developing a HPLC method which is stability indicating. The method was validated according to ICH Q2 (R1) guideline.

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