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A validated chiral LC method for enantiomeric separation of levocetirizine using protein based chiral stationary phase

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ABSTRACT

A rapid isocratic chiral LC method has been developed for the separation of (S)-Cetirizine (Dextrocetirizine) and (R)-(-)-Cetirizine (Levocetirizine). Good resolution with $R_s > 1.5$ was obtained using protein based chiral stationary phase, ES-OVM column (150 x 4.6 mm, 5 μ m particle size) and 20mM potassium dihydrogen orthophosphate (pH 7.0) buffer, Acetonitrile (87:13,v/v) as the mobile phase at ambient temperature. Flow rate was kept at 0.6 mL min⁻¹ and elution was monitored by UV detection at 230 nm. This method allowed for the detection and quantification of R-Cetirizine levels at 0.056 and 0.182 μ g mL⁻¹ respectively. The method was validated by following ICH guidelines. © 2011 Trade Science Inc. - INDIA

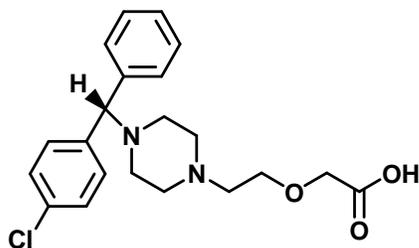
KEYWORDS

Liquid chromatography;
Enantiomer;
Method validation;
Chiral Stationary Phase;
Resolution.

INTRODUCTION

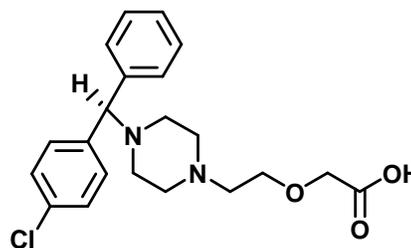
(S)-Cetirizine (Dextrocetirizine) (Figure 1) and (R)-(-)-Cetirizine (Levocetirizine) (Figure 2) is a second-generation antihistamine cetirizine. Levocetirizine (as levocetirizine dihydrochloride) is a third-generation non-sedative antihistamine, developed from the second-generation antihistamine cetirizine. Chemically, levocetirizine

is the active enantiomer of cetirizine. It is a potent histamine H-1 receptor antagonist with anti-inflammatory and antiallergic properties. It is the R-enantiomer of the cetirizine racemate. Levocetirizine works by blocking histamine receptors. It does not prevent the actual release of histamine from mast cells, but prevents it binding to its receptors. This in turn prevents the release of other allergy chemicals and increased blood supply to



2-[2-[4-[(S)-(4-chlorophenyl)-phenyl-methyl] piperazin-1-yl] ethoxy] acetic acid

Figure 1 : Chemical structures of (S)-Cetirizine (Dextrocetirizine)



2-[2-[4-[(R)-(4-chlorophenyl)-phenyl-methyl] piperazin-1-yl] ethoxy] acetic acid

Figure 2 : Chemical structures of (R)-(-)-Cetirizine (Levocetirizine)

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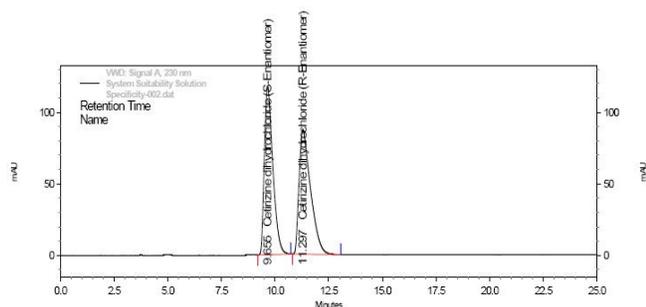


Figure 3 : Typical chromatogram of (S)-Cetirizine (Dextrocetirizine) and (R)-(-)-Cetirizine (Levocetirizine)

the area, and provides relief from the typical symptoms of hay fever. The review analyses the levocetirizine's properties in terms of safety and efficacy both in allergic rhinitis and urticarioid syndromes^[1].

Indian Pharmacopeia reported a normal phase chiral HPLC method for determination of the enantiomer composition of Levocetirizine dihydrochloride by using a chiralpak AD-H column. The enantiomers were resolved ($RS = 0.96$) by use of n-hexane:2-propanol:Ethanol:Trifluoro-acetic acid, 70:15:15:0.02 (v/v), as mobile phase. Analysis time was over 30 min, however. The objective of this investigation was to develop and validate a simple and reliable reversed phase HPLC method for enantioselective pharmaceutical analysis of Levocetirizine dihydrochloride^[2].

The first method was based on the enantioseparation of cetirizine on silica gel TLC plates using different chiral selectors as mobile phase additives. The mobile phase enabling successful resolution was acetonitrile-water 17:3, (v/v) containing 1 mM of chiral selector, namely hydroxypropyl-beta-cyclodextrin, chondroitin sulphate or vancomycin hydrochloride. The second method was a validated high performance liquid chromatography (HPLC), based on stereoselective separation of cetirizine and quantitative determination of its eutomer (R)-levocetirizine on a monolithic C18 column using hydroxypropyl-beta-cyclodextrin as a chiral mobile phase additive^[3].

Separation of enantiomers has become very important in analytical chemistry, particularly in the pharmaceutical and biological fields, reflecting the fact that different enantioselective properties can impact on pharmacokinetics and result in a variety of different pharmacological or toxicological effects^[4-7]. As a consequence, regulatory authorities insist on stringent investi-

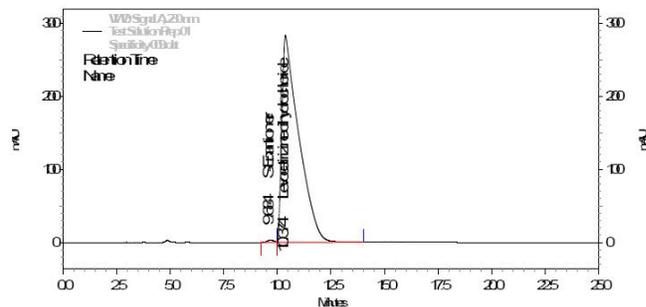


Figure 4 : Typical chromatogram of (R)-(-)-Cetirizine (Levocetirizine)

gations when evaluating the safety and the effectiveness of chiral drugs. This places a significant importance on the application of enantiomeric separations to all stages of drug development and the commercialization process. The development of new methods for efficient chiral separations are mainly based on high performance liquid chromatography (LC), capillary electrophoresis (CE) or gas chromatography (GC). A large number of commonly employed LC methods are based on the use of chiral stationary phases (CSPs).

As per the requirement of various regulatory authorities, chiral method development for Levocetirizine is very essential. In this study, a simple, cost-effective and efficient chiral LC method was developed and validated as per the ICH guidelines^[8,9].

EXPERIMENTAL

Chemicals and reagents

Levocetirizine dihydrochloride is (R)-2-[2-[4-[(4-Chlorophenyl) phenyl methyl] piperazin-1-yl] ethoxy] acetic acid dihydrochloride, was obtained from our research and development department (Hyderabad, India). HPLC-grade acetonitrile was purchased from Runa Chemicals Pvt.Ltd (Maharashtra, India). Potassium Dihydrogen Orthophosphate and Triethylamine were purchased from Merck (Mumbai, India). HPLC water was from a Milli-Q system (Milli-pore, Bedford, MA, USA). Other chemicals were of analytical grade.

Instrumentation and chromatographic conditions

The resolution of the enantiomers was performed on 1200 Series HPLC system (Agilent Technologies, Waldron, Germany) equipped with a DE62964377 quaternary pump, a JP73063462 degasser, a

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DE64766538 autosampler, a DE63068058 thermostatted column compartment, and a DE71361342 UV-detector; data were processed by use of EZChrome Elite software. The column used for the analytical separation was protein based column having ovomucoid as a stationary phase coated on silica gel known as ES-OVM (150 x 4.6 mm, 5 μ m) procured from Shinwa chemical industries, LTD (Japan).

The mobile phase consisted of 20mM potassium dihydrogen orthophosphate (pH 7.0) buffer, Acetonitrile (87:13,v/v) at ambient temperature. Flow rate was kept at 0.6 mL min⁻¹ and the sample injection volume was 10 μ L.

The appropriate wavelength for the detection of enantiomers was determined by wavelength scanning over the range of 200-400nm using Agilent 1200 series photodiode array detector and the chromatograms were monitored by UV detection at a wavelength of 230nm.

Preparation of solutions

A stock solution of racemic mixture was prepared at 200 μ g mL⁻¹ by dissolving appropriate amount in the mobile phase as diluent. Standard Levocetirizine dihydrochloride stock solution was prepared having concentration 500 μ g mL⁻¹ using mobile phase as diluent. Further dilution to a concentration of 10 μ g mL⁻¹ was made using the same diluent. This solution was used as diluted standard^[10].

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

In order to get optimum resolution and selectivity for the two enantiomers (S)-Cetirizine (Dextrocetirizine) and (R)-(-)-Cetirizine (Levocetirizine) various experiments were conducted by using various CSPs containing protein and cellulose derivatives. Enantiomeric separation is known to be achieved by the formation of transient diastereomeric complexes, mostly based on hydrogen bonding, dipole-dipole and π - π interactions^[11].

Chiral AGP (α 1-acid glycoprotein) is a plasma protein containing 181 amino acids and 14 sialic acid residues. The carbohydrate moiety of the AGP is believed to be involved in binding of basic compounds at physiological pH. Chiral separation with AGP is conducted under reverse phase conditions and the mobile phase is

usually a mixture of aqueous buffer and organic modifier. Different buffer such as Potassium dihydrogen orthophosphate, Sodium perchlorate, Potassium hexafluoro phosphate and Ammonium acetate used as mobile phases were not successful and Both isomers were separated by using Potassium dihydrogen orthophosphate buffer with Acetonitrile but tailing and broadening of peaks were observed, Although separation was substantial the AGP column was not considered further because of poor peak shape and the long run time (more than 50 min).

The Chiralcel OD-RH column has a 3, 5-diethylphenylcarbamate derivative of cellulose adsorbed on silica gel. By using this column proper resolution was not achieved by using the buffer and organic modifiers.

The Ultron ES-OVM column is a protein based column having ovomucoid as a stationary phase coated on silica gel. Best separation was obtained by using of Potassium dihydrogen orthophosphate as buffer. The identity of the organic mobile phase modifier has large effect on the retention times of the enantiomers and their separation. Because small variations of the concentration of acetonitrile in the mobile phase effected both retention time and enantio selectivity, So methanol was selected as organic modifier. Methanol is more viscous than acetonitrile and known to cause to more column pressure, so finally acetonitrile was selected as organic modifier. The effect of mobile phase pH was studied in the range of 6.0–7.5 and mobile phase pH was shown to have a negligible effect on retention and selectivity. Separation of Cetirizine dihydrochloride enantiomers was studied using potassium dihydrogen orthophosphate solutions of different concentration (10mM, 20mM, and 100mM) at the same pH. Although retention times of the Cetirizine dihydrochloride enantiomers were slightly effected when higher concentrations of potassium dihydrogen orthophosphate were used, increasing the ionic strength of the mobile phase had no effect on enantioselectivity but effected some what in peak shape which is not recommendable.

The best separation of the enantiomers of Cetirizine dihydrogen chloride was achieved on the Ultron ES-OVM column with 20mM potassium dihydrogen orthophosphate (pH 7.0): acetonitrile, 87:13 (v/v) as mobile phase with flow rate of 0.6 mL min⁻¹. Hence,

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the chiral separation was optimized using isocratic conditions as these offers more rapid analysis attributable to the presence of column re-equilibration steps. The typical retention times of (S)-Cetirizine (Dextrocetirizine) and (R)-(-)-Cetirizine (Levocetirizine) were approximately 9 and 11 min, respectively.

Limit of detection and quantification

The lowest LOD and the LOQ were determined based on signal-to-noise ratios using analytical responses of 3 and 10 times to the background noise, respectively^[12]. The LOD and the LOQ for (R)-(-)-Cetirizine (Levocetirizine) were calculated to be 0.056 and 0.182 $\mu\text{g mL}^{-1}$, respectively.

Linearity

Under the optimized working conditions, plotted

standard calibration curve for (R)-(-)-Cetirizine (Levocetirizine) was linear over the concentration range of 10 $\mu\text{g mL}^{-1}$ (LOQ) to 15 $\mu\text{g mL}^{-1}$. The results of the statistical analysis of the experimental data, such as slope, the intercept and the correlation efficient obtained by the least squares treatment of the results were 554802, -158483 and 0.996, respectively. The standard calibration curve was linear with the linear regression equation $y = 554802 - 158483x$.

Precision

The repeatability (intra-day) of the method was evaluated by the determination of peak area percentage RSD of (R)-(-)-Cetirizine (Levocetirizine) for six replicate injections of diluted standard at the levels of 0.182 $\mu\text{g mL}^{-1}$ (LOQ) and 15 $\mu\text{g mL}^{-1}$. The results were summarized in the TABLE 1.

TABLE 1 : Results of precision at LOQ

S.No.	Parameter	%RSD for RT of Levocetirizine	%RSD for % Area of Levocetirizine	%RSD for RT of Dextrocetirizine	%RSD for % Area of Dextrocetirizine
1	Method Precision	0.257	0.39	0.71	0.33
2	Precision at LOQ	0.30	2.11	NA	NA

NA: Not Applicable

Robustness

The Robustness of the method was studied by varying number of method parameter. The experimental conditions were deliberately varied in order to determine the impact on resolution of enantiomers. The flow rate on resolution was studied by varying the flow rate by $\pm 0.1 \text{ mL min}^{-1}$. The pH effect was studied by varying ± 0.2 units. In addition, the percentage of acetonitrile in the mobile phase was varied $\pm 10\%$. No significant change observed in the resolution of enantiomers, results were shown in the TABLE 2, illustrating the robustness of the developed method.

TABLE 2: Results of Robustness study

Parameter	Variation	USP resolution (Rs)
Flow rate	0.5 mL min^{-1}	2.02
	0.7 mL min^{-1}	1.85
pH	6.8	1.85
	7.2	1.93
Temperature	23 $^{\circ}\text{C}$	1.99
	27 $^{\circ}\text{C}$	1.90
Organic ratio	12 mL Acetonitrile	1.95
	14 mL Acetonitrile	1.71

CONCLUSION

An isocratic enantioselective HPLC method that enables sensitive determination of (S)-Cetirizine (Dextrocetirizine) and (R)-(-)-Cetirizine (Levocetirizine) was developed. The method was found to be simple, sensitive, precise, and robust. Hence, this method can be used in quality control laboratories for the routine analysis.

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