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Sensitive HPLC method for determination of related substance of levocetirizine dihydrochloride in solid oral formulations

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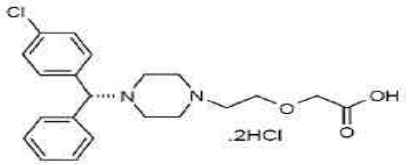
ABSTRACT

A sensitive high performance liquid chromatography method has been developed and validated for the determination of Levocetirizine dihydrochloride related substances in solid oral formulation. Acetonitrile and diluted Sulphuric acid was used as a mobile phase for the elution and separation of Levocetirizine dihydrochloride and its related substance. Lichrocarrt Si60, 250 X 4.0, 5µ (Cartridge column) was used at 0.8 ml/min flow rate to achieve good resolution of the analyte and its impurities. Levocetirizine dihydrochloride was exposed to various stress conditions such as acid, base, oxidation & thermal degradations along with Placebo & Formulation. But it was found to be stable under all stress conditions. The detector linearity was established from concentrations ranging from 0.06 µg/ml to 0.60µg/ml for Levocetirizine dihydrochloride and 0.03 to 0.30 µg/ml for its impurities with a correlation co-efficient of 0.9999.The LOD and (LOQ) found to be in a range of 0.01 µg/ml and 0.06µg/ml for Levocetirizine dihydrochloride and 0.005 µg/ml and 0.03 µg/ml for related substances respectively. Sample Solutions stability was found stable for than 42 hours. The molecule was found stable in all the stress conditions and proved to be robust with respect to changes in flow rate and buffer composition. The proposed method is found to be sensitive, precise, rapid and reproducible.

Key words: HPLC, Levocetirizine, Related substance, Validation.

INTRODUCTION

Levocetirizin dihydrochloride is an orally active H1-receptor antagonist. The chemical name is (R)-[2-[4-[(4-chlorophenyl) phenyl methyl]-1-piperazinyl] ethoxy] acetic acid dihydrochloride. Levocetirizine dihydrochloride is the R enantiomer of cetirizine hydrochloride, a racemic compound with antihistaminic properties. The empirical formula of levocetirizine dihydrochloride is $C_{21}H_{25}ClN_2O_3$•2HCl. The molecular weight is 461.82 and the chemical structure is shown below [1]:



The antihistaminic activity of Levocetirizine has been documented in a variety of animal and human models [1, 2, 3, 4, 5]. In vitro binding studies revealed that Levocetirizine has an affinity for the human H_1-receptor 2-fold higher than that of cetirizine (Ki = 3 nmol/L *vs*. 6 nmol/L, respectively). The clinical relevance of this finding is unknown. The extent of metabolism of Levocetirizine in humans is less than 14% of the dose and therefore differences resulting from genetic polymorphism or concomitant intake of hepatic drug metabolizing enzyme inhibitors are expected to be negligible. Metabolic pathways include aromatic oxidation, N- and O-dealkylation, and taurine conjugation. Dealkylation pathways are primarily mediated by CYP 3A4 while aromatic oxidation involves multiple and/or unidentified CYP isoforms. A number of studies have been carried out for the determination of Levocetirizine in human Plasma & solid formulation. This research article reports a precise, accurate and sensitive HPLC determination method with UV detection, useful for routine quality control of Levocetirizine dihydrochloride and its related substances in solid oral formulation [6]. The method was validated by parameters such as linearity, accuracy, precision and robustness. Experimental design was used for validation to evaluate the robustness and intermediate precision [7, 8].

MATERIALS AND METHODS

Chemicals

Levocetirizine dihydrochloride (purity -100%) and the impurities of [(RS)-1-[(4-chlorophenyl)phenyl methyl] piperazine (Impurity A)], [(RS)-2-[4-[(4-chlorophenyl)phenyl methyl]piperazin-1-yl]acetic acid (Impurity B)], [RS)-2-[2-[4-[(2-chlorophenyl)phenyl methyl]piperazin-1-yl]ethoxy]acetic acid (Impurity C)], [1,4-bis[(4-chlorophenyl)phenyl methyl] piperazine (Impurity D)] and [2-[4-[(RS)-(4-chlorophenyl) phenyl methyl] piperazin-1-yl]ethanol (Impurity G)], Sulphuric Acid- (Merck limited, India) and Acetonitrile (Rankem, India). All the other chemicals and solvents were used of analytical grade and HPLC grade.

Apparatus

The analysis was carried out on Waters Alliance HPLC systems 2695 separation module connected to 2996 Photo diode array detector. Data acquisition was carried out using Empower -2 software. Several columns were used for optimizing the chromatographic condition. The parameters being focused are resolution of Levocetirizine and its related impurities and column life.

1. Luna-C 18 (250mm×4.6mm), 5µ. (Make - Phenomenex).
2. Inertsil ODS 3V (250mm×4.6mm) 5µ, (Make-GL Sciences Inc Japan).
3. Hypersil ODS-, 250 X 4.6mm, 5µ, (Make-Thermo).
4. Lichrocarrt Si60 -, 250 X 4.0 mm, 5µ column, (Make- Merck)

Chromatographic conditions

The separation of Levocetirizine and its related substances were achieved by isocratic method using dilute Sulphuric acid (Dissolved accurately 0.15 ml of Sulphuric acid in 100 ml) as buffer, and Acetonitrile. Components of Mobile phase such as dilute Sulphuric acid and acetonitrile were taken in the ratio of 7: 93 respectively at a flow rate of 0.8 ml/minute [9, 10]. Detection and purity establishment of the main drug and the related substances were achieved by using a photo diode array (PDA) detector at 230nm [11]. The standard and test samples were prepared by using mobile phase as diluent to achieve desired working concentration. A volume of 20µl of the sample was injected to chromatographic system as the injection volume. The optimized run time was found to be 35 minutes (Enantiomeric impurity are not included in this method).

Standard preparation

Standard stock solution (200µg/mL) of Levocetirizine and its impurity was prepared by using mobile phase as diluent. The working standards were diluted to volume with diluent to achieve a final working concentration. A system suitability test was performed for six replicate standard injections.

Sample preparation

The drug was extracted from the tablet formulation of 5.0 mg label claim by using the mobile phase as diluent. Sample was diluted with mobile phase to achieve final working concentration.

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Table 1: Effect of diluted Sulphuric acid on RT (separation)

% Buffer Composition	Impurity A	Impurity B	Impurity C	Impurity D	Impurity G	Levocetirizine Dihydrochloride
10 %	32.67	17.67	18.10	11.94	28.62	20.0
8 %	29.41	14.52	16.04	8.74	25.44	17.84
7 %	27.52	12.36	13.91	6.78	23.58	15.67
5 %	25.12	10.22	12.34	4.44	21.12	13.14
4 %	21.97	6.10	8.23	2.54	18.03	9.64

Table 2: Peak purity of Levocetirizine dihydrochloride in stressed condition

Stress condition	% Degradation	Purity angle	Purity threshold	Flag
Acid degradation	Nil	0.283	0.376	No
Base degradation	Nil	0.389	0.560	No
Peroxide degradation	Nil	0.312	0.498	No
UV light degradation	Nil	0.432	0.685	No
Heat degradation	Nil	0.342	0.466	No

Table 3: System suitability parameters

Sr. No	Retention Time	USP Tailing	USP Resolution	Theoretical plates	Purity angle	Purity Threshold
Levocetirizine dihydrochloride	15.67	1.10	NA	13881	0.379	0.587
Impurity A	27.52	1.16	15.80	9745	0.254	0.378
Impurity B	12.36	1.24	4.12	12738	0.417	0.475
Impurity C	13.91	1.20	3.64	10785	0.339	0.582
Impurity D	6.78	1.32	17.31	12564	0.487	0.663
Impurity G	23.58	1.16	12.34	15679	0.321	0.564

Table 4: Linearity of Levocetirizine and impurities from LOQ level to 150%

% Concentration	Concentration in µg/ml	Area response
LOQ	0.064	556
50%	0.2	2347
80%	0.32	3868
100%	0.4	4832
120%	0.48	5895
150%	0.6	7428

Table 5: Assay value of six preparations of drug samples

Sample No	% Assay	Total impurities
Sample 1	99.68	0.25
Sample 2	100.02	0.25
Sample 3	98.86	0.26
Sample 4	99.54	0.24
Sample 5	101.10	0.25
Sample 6	100.25	0.26
Mean	99.91	0.25
SD	0.753	0.008
% RSD	0.75	2.99

Table 6: Accuracy for Levocetirizine and related substances

Level	% Recovery Recovery Imp-A	Recovery Imp-B	Recovery Imp-C	Recovery Imp-C	Recovery Imp-C	Levocetirizine dihydrochloride
LOQ Level	102.96	98.36	96.65	100.23	98.67	105.49
50 %	99.65	97.34	98.62	99.63	99.47	101.26
100 %	100.13	100.56	99.78	102.34	100.35	99.86
150 %	101.83	101.32	102.67	103.61	102.34	99.25

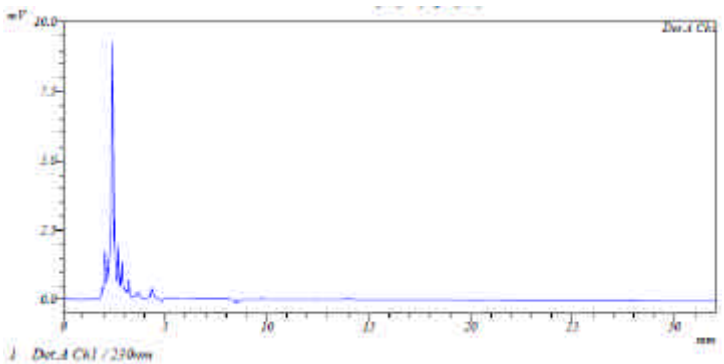


Figure 1 (a): Placebo chromatogram

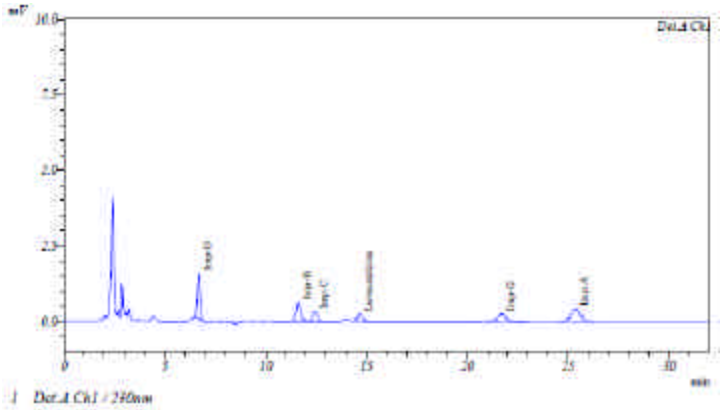


Figure 1 (b): Spiked Chromatogram (Levocetirizineand impurities)

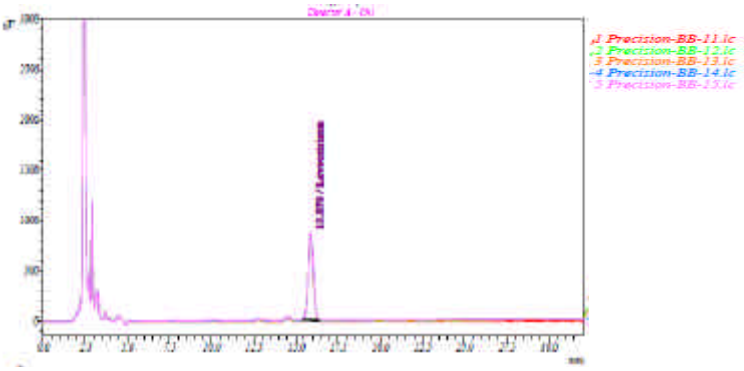


Figure 1 (c): Standard Chromatogram (Levocetirizineand impurities)

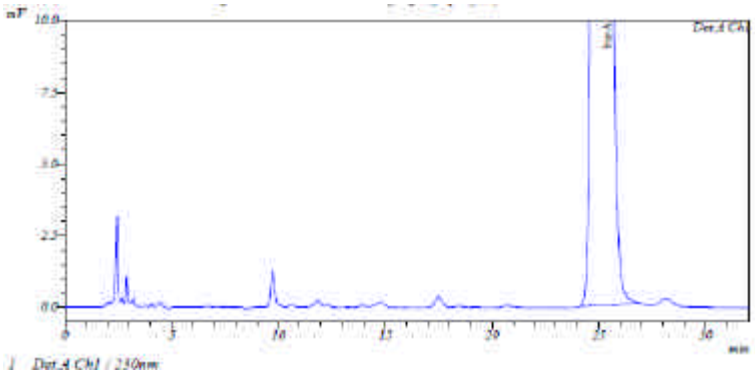


Figure 1 (d): Impurity-A Chromatogram

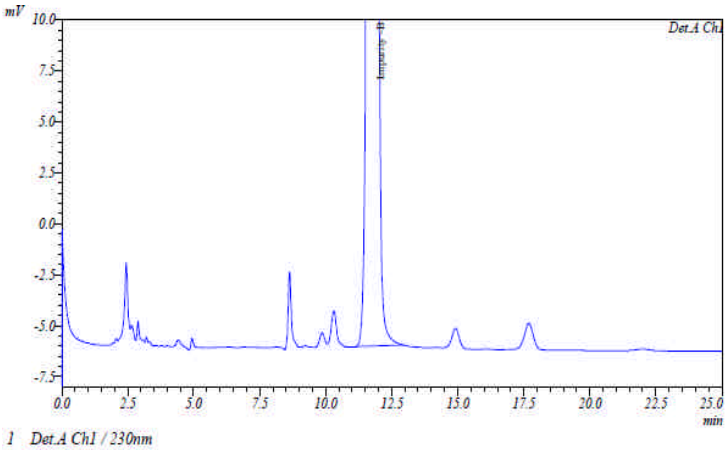


Figure 1 (e): Impurity-B Chromatogram

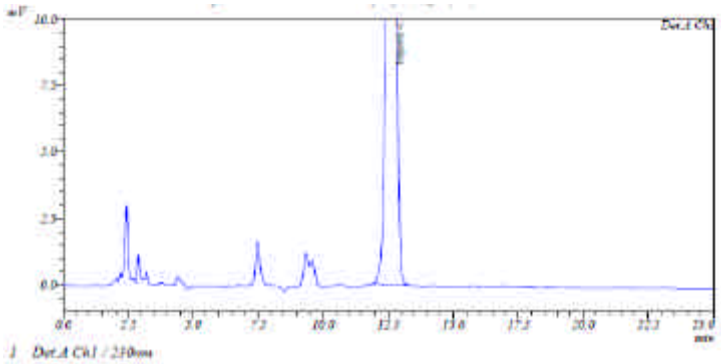


Figure 1 (f): Impurity-C Chromatogram

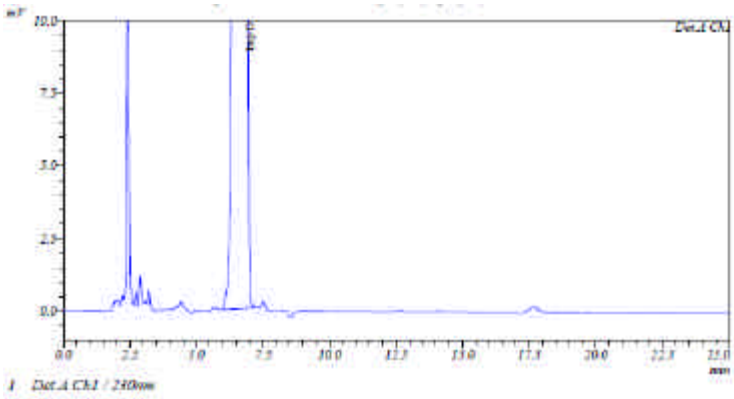


Figure 1 (g): Impurity-D Chromatogram

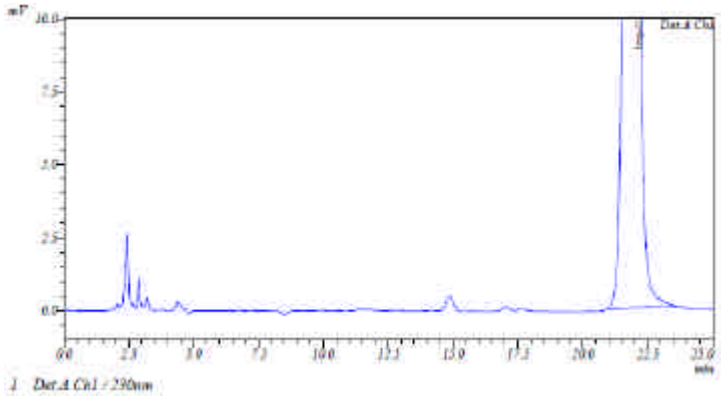


Figure 1 (h): Impurity-G Chromatogram

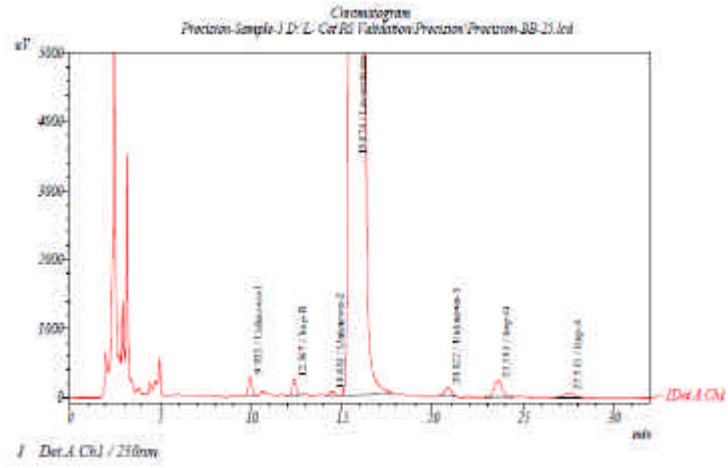
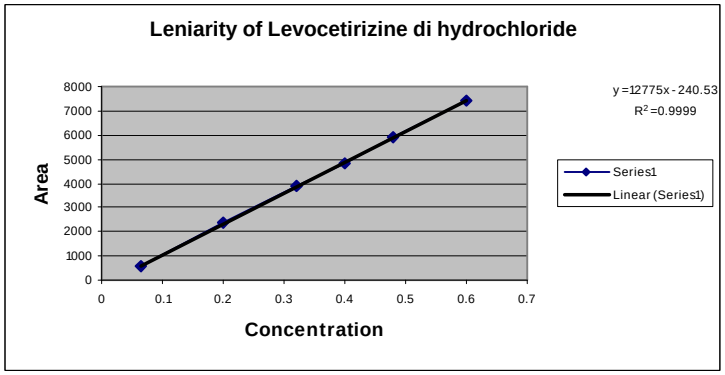
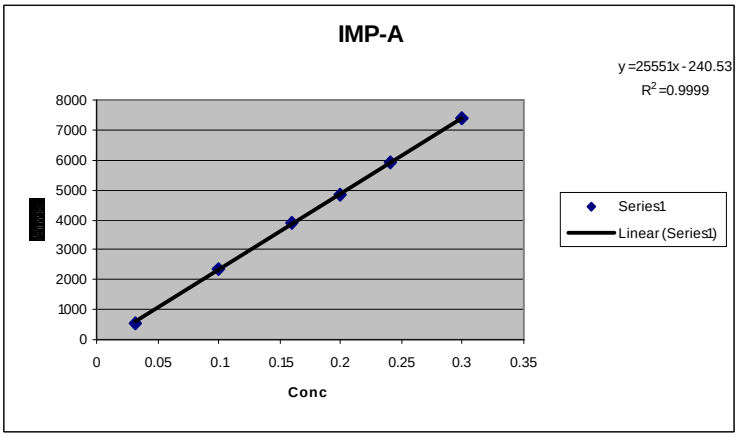


Figure 2: Sample chromatogram



Graph 1(a): Linearity of Levocetirizine dihydrochloride



Graph 1(b): Linearity of Levocetirizine dihydrochloride impurity -A

Buffer selection

Different buffers such as Phosphate buffer (sodium/potassium), Acetate buffer and diluted Sulphuric acid buffer were evaluated for system suitability parameters and over all chromatographic performance. In the sequential trials carried out using different buffers it was concluded that diluted Sulphuric acid was found to be suitable for effective separation of Levocetirizine dihydrochloride peak and its impurities. The concentration of diluted Sulphuric acid effect on the retention time and separation peak asymmetry of Levocetirizine dihydrochloride and its related compounds details are tabulated in Table 1.

Effect of organic modifier

The usage of organic solvents like acetonitrile gave a good chromatographic picture with increased resolution. Introduction of organic solvent has shown good theoretical plate and asymmetry factor for different impurities.

RESULTS AND DISCUSSION

Specificity

The Forced degradation of active substance, placebo and formulation was carried out for acid degradation, base degradation, oxidation and thermal degradation. The acid, base and oxidation stress studies were carried out by refluxing active substance for 24 hours with 5 ml 1N hydrochloric acid, 1N sodium hydroxide and 30% hydrogen peroxide respectively and thermal degradation was carried out by heating the drug powder at 80°C for about 24 hrs. The drug and the formulation were found to be stable under all the stress conditions. All the stress conditions samples injected with replicate injections of Levocetirizine dihydrochloride, spiked with all impurities. The system deemed to be suitable as tailing factor =2.0, theoretical plate > 2000, and resolution between closely eluting impurity >1.5 results obtained [6, 7]. The results obtained are tabulated in tables 2, 3 and figures 1(a) to 1(h) and figure 2.

Linearity

The linearity solutions were prepared by using acetonitrile and mobile phase as diluent. Analyte solution has shown linear response for concentration levels ranging from 0.06µg/ml to 0.6µg/ml for Levocetirizine dihydrochloride and 0.005µg/ml to 0.03µg/ml related substances respectively. The correlations co-efficient value was found to be 0.9999. LOD and LOQ of Levocetirizine all

impurities were calculated by signal to noise ratio method results are tabulated in table 4 and graph 1(a) to 1(b).

Precision

The method was found to be precise with six sample preparations for the quantification of Levocetirizine dihydrochloride and its impurities. Impurity solution spiked to the sample concentration containing Levocetirizine. The % relative standard deviation (RSD) of Levocetirizine and its total impurity in six sample preparation was found to be = 2.0 % for assay and = 5.0 % for impurity. Obtained results are tabulated in table 5 and figures 2.

Accuracy

The recovery of Levocetirizine and impurities-A, B, C, D and G were determined by spiking each impurity at four different levels starting from LOQ to 150%. The recovery range for all impurities was found to be between 96% - 106% with RSD between 0.65% - 2.87 %. Obtained results for accuracy study are tabulated in table 6.

Solution stability

The solution stability of the standard, impurities and test solution prepared in mobile phase was studied for 42 hr at bench top. The solution under study was compared with freshly prepared standard solution. Results obtained showed that the samples were found to be stable for period of 42 hours.

Robustness

The robustness was carried out by varying the conditions with reference to change in flow rate, and buffer composition. The study was conducted at different flow rates of 0.7 ml/min, and 0.9 ml/min. The mobile phase buffer composition was modified to 6% & 8% to study the effect of buffer composition. Standard solution with six replicate injections, resolution solution containing the mixture of all the impurities and sample solution were injected. The method was found to be robust with respect to flow rate and buffer composition with out any changes in system suitability parameters such as tailing, resolution and theoretical plate.

CONCLUSION

The chemistry of column employed in present method allows working with 100% aqueous mobile phase showing excellent chromatographic features with respect to Levocetirizine dihydrochloride and its related substances. The method also provides selective quantification of Levocetirizine and impurities without interference from blank and placebo, thereby affirming stability- indicating nature of method. The proposed method is highly sensitive, reproducible, and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method was robust in the separation and quantification of Levocetirizine dihydrochloride and its related substances. This method can be used for the routine analysis of commercial samples. The information presented herein could be very useful for quality monitoring of bulk samples and as well employed to check the quality during stability studies.

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