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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LEVOCETRIZINE DIHYDROCHLORIDE, METHYLPARABEN, AND PROPYLPARABEN IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A stability-indicating new simple, rapid, selective, precise and accurate isocratic reverse phase–high performance liquid chromatography (RP–HPLC) method was developed and validated for the estimation of Levocetrizine dihydrochloride in oral solution. The chromatographic separations were achieved using a High performance liquid chromatography (Waters symmetry C8, 250 x 4.6 mm, 5µ) column, employing solvent mixture –I and solvent mixture-II in the ratio of 60:40%v/v as mobile phase with 1.0 mL/min flow rate. The column temperature was maintained at 30°C and a detector wavelength of 230 nm was employed. Levocetrizine dihydrochloride was subjected to various stress conditions like acidic, basic, thermal and photolytic degradations. The method was validated in accordance with ICH guidelines with respect to linearity, accuracy, precision, specificity. The method results in excellent separation between the drug substance and its stress-induced degradation products. Linearity was found to be in the range of 12-75 ppm with significantly high value of correlation coefficient. Regression analysis showed r^2 0.999. The accuracy of the method was proved; the mean recovery of Levocetrizine was 97.6% to 98.8%. The method was statistically validated and RSD was found to be less than 2.0% indicating high degree of ruggedness and precision of the proposed HPLC method. The validated method can be used in routine quality control analysis of oral solution containing Levocetrizine dihydrochloride. The proposed stability-indicating method proved to be a simple, sensitive, accurate, precise, reproducible method for the estimation of Levocetrizine dihydrochloride.

Keywords: Levocetrizine, RP HPLC, ICH Guide lines, Degradation, Method Validation.

INTRODUCTION

Levocetrizine, chemically 2-[2-[4-[(R)-(4-chlorophenyl)- phenyl-methyl] piperazin-1-yl] ethoxy] acetic acid[1]. It is a third-generation non-sedative antihistamine and used in the form of levocetrizine dihydrochloride for the treatment of allergic rhinitis and chronic idiopathic urticaria[2]. It is an active R-enantiomer of cetirizine, orally active, potent, and selective and long acting H1-histamine receptor antagonist with no anticholinergic activity[3] .Levocetirizine dihydrochloride is official in IP[4]. Present study involves development of a convenient, rapid, and cost efficient and user friendly reversed-phase (RP)-HPLC method with a simple and easily available mobile phase for quantitative estimation of Levocetrizine dihydrochloride, Methylparaben, and Propylparaben. The

optimized method was developed and validated as per International Conference on Harmonisation (ICH) guidelines. Several analytical methods have been reported for the analysis of Levocetrizine alone or in combination with other drugs such as few UV/Vis spectrophotometric methods[5-9], TLC method[10], HPLC methods[11-22], several bioanalytical methods have been described for the analysis of levocetirizine in plasma or urine using liquid chromatography-tandem mass spectroscopic (LC-MS/MS) method[23-27], and gas chromatographic (GC) method[28]

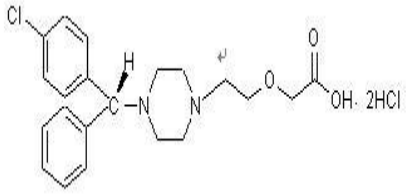

Fig. 1: Chemical structure of Levocetirizine dihydrochloride

MATERIALS AND METHODS

The reference samples of Levocetirizine were provided as gift samples from Ranbaxy Laboratories Limited, Gurgaon, Ammonium Di Hydrogen Phosphate (EMerck,USA), Triethylamine (Qualigens, India), Sodium-1-Octane Sulphate (Merck, India), Tetrahydrofuran (Qualigens, India), Isopropyl Alcohol (Qualigens, India), and HPLC grade methanol (Merck, India), were used. Millipore purified water was further filtered through a 0.45-µ membrane filter (Durapore, Millipore) to provide Milli-Q water.

Instrument and chromatographic conditions

The HPLC system used for the method development and validation consisted Alliance-Waters 2695 separation module with Waters 2996 photo diode array detector equipped with EMPOWER software. The column used for separation of analytes is Waters symmetry C8, 250 x 4.6 mm, 5µ, at 30°c. Mobile phase consisting of solvent Mixture-I and solvent Mixture-II in the ratio of 60:40%v/v with a flow rate of 1.0 mL/min. It was filtered through 0.45µm nylon filter and sonicated for 15 minutes in ultrasonic bath. Sample was analyzed at 230 nm with an injection volume of 20 µL and runtime 25min.

Preparation of Buffer

Weighed accurately 4.6g of Ammonium dihydrogen phosphate and dissolved it in 1000ml of Milli-Q water. Adjusted the pH to 6.0 with Triethyl amine, and added 500mg of Sodium-1-octane sulphonate and sonicated to dissolve, filtered through 0.45µm nylon membrane filter and degassed.

Preparation of solvent Mixture-I

Mixed Buffer and Tetra hydrofuran in the ratio of 90:10%v/v

Preparation of solvent Mixture-II

Mixed Methanol and Isopropyl alcohol in the ratio of 75:25% v/v

Preparation of Mobile phase

Prepared the degas mixture of solvent mixture-I and solvent mixture-II in the ratio of 60:40%v/v.

Preparation of Diluent

Prepared a degassed mixture of buffer and methanol in the ratio of 70:30%v/v

Levocetirizine Standard stock Preparation

Weighed and transferred accurately about 50 mg of of Levocetirizine dihydrochloride Working Standard into a 100 ml clean dry volumetric flask, added about 60 ml of Diluent, sonicated for 5 minutes, and diluted to volume with Diluent.

Methyl paraben Standard stock Preparation

Weighed and transferred accurately about 34 mg of Methyl paraben Working Standard into a 50 ml clean dry volumetric flask, added about 20 ml of diluent, sonicated for 5 minutes, and diluted to volume with Diluent.

Propyl paraben Standard stock Preparation

Weighed and transferred accurately about 19 mg of Propyl paraben Working Standard into a 50 ml clean dry volumetric flask, added about 20 ml of Diluent, sonicated for 5 minutes, and diluted to volume with Diluent. Diluted 5ml of standard stock solution to 25 ml with Diluent and mix

Preparation of Standard solution of Levocetirizine solution

Pipetted out 5 ml of Levocetirizine standard stock solution, 5ml of Methyl paraben Standard stock solution and 5ml Propyl paraben Standard stock solution into a 50ml Volumetric flask and added 35ml of diluent and sonicated for 15minutes and then diluted to 50 ml with diluent.

Sample preparation
Accurately transferred 5ml of Levocetirizine oral solution into 50ml volumetric flask. Added about 30ml of diluent and sonicated for 15minutes and then diluted with diluent.

METHOD VALIDATION
The HPLC method was validated to show precision, specificity, linearity, accuracy, ruggedness, robustness and stability, in analytical solution. Validation of the developed method was carried out as per ICH and FDA requirements

SYSTEM SUITABILITY AND SYSTEM PRECISION
A Standard solution was prepared by using Levocetirizine, Methylparaben and Propylparaben working standards as per test method and was injected six times into the HPLC. System suitability parameters were evaluated from standard chromatograms by calculating the % RSD of six replicate injections for Levocetirizine, Methylparaben and Propylparaben (Table 1).

Method precision
The precision of test method was evaluated by analyzing six samples of same batch by the proposed method. Data shown in Table 2 indicate an acceptable level of precision.

Ruggedness
The ruggedness of method was verified by conducting the precision study by using different HPLC, different columns of same make by different analyst on different day. Six samples of same batch were prepared and analysed by the proposed method. The mean, standard deviation, and %RSD for the two sets of data are shown in Table 3. Ruggedness of the method is indicated by the overall RSD between the two sets of data.

Table 1: Results of System precision

Injection No.	Levocetirizine	Methyl Paraben	PropylParaben
	Area counts (uv*sec)	Area counts (uv*sec)	Area counts (uv*sec)
1	5412895	243915	281639
2	5380214	243950	283677
3	5396184	243936	283733
4	5348129	244041	283887
5	5341765	244154	282749
6	5375837	243999	282887
Mean	5375837.3	243999.2	283095.3
SD	27331.62399	88.56053	855.0962
RSD (%)	0.51	0.04	0.30

Table 2: Results of Method precision

Samples No.	Levocetirizine	MethylParaben	PropylParaben
	% Assay	% Assay	% Assay
1	99.6	98.9	98.5
2	97.8	99.6	100.2
3	100.3	97.8	99.3
4	99.8	100.1	99.4
5	98.9	98.8	98.6
6	99.2	99.5	100.3
Mean	99.3	99.1	99.4
SD	0.86641	0.803534	0.762671
RSD (%)	0.87	0.81	0.77

Table 3: Results of Ruggedness

Samples No	Levocetirizine % Assay		Methyl Paraben % Assay		Propyl Paraben % Assay	
	Set-I	Set-II	Set-I	Set-II	Set-I	Set-II
1	99.6	99.3	98.9	98.2	98.5	99.8
2	97.8	98.5	99.6	98.6	100.2	99.1
3	100.3	99.6	97.8	99.5	99.3	98.6
4	99.8	100.2	100.1	99.4	99.4	99.2
5	98.9	99.7	98.8	99.8	98.6	100.1
6	99.2	98.9	99.5	98.9	100.3	99.4
Mean	99.3	99.4	99.1	99.1	99.4	99.4
SD	0.87	0.61	0.80	0.61	0.76	0.53
RSD (%)	0.87	0.61	0.81	0.61	0.77	0.54
Overall Mean	99.3		99.1		99.4	
Overall SD	0.71		0.68		0.63	
Overall RSD (%)	0.72		0.69		0.63	

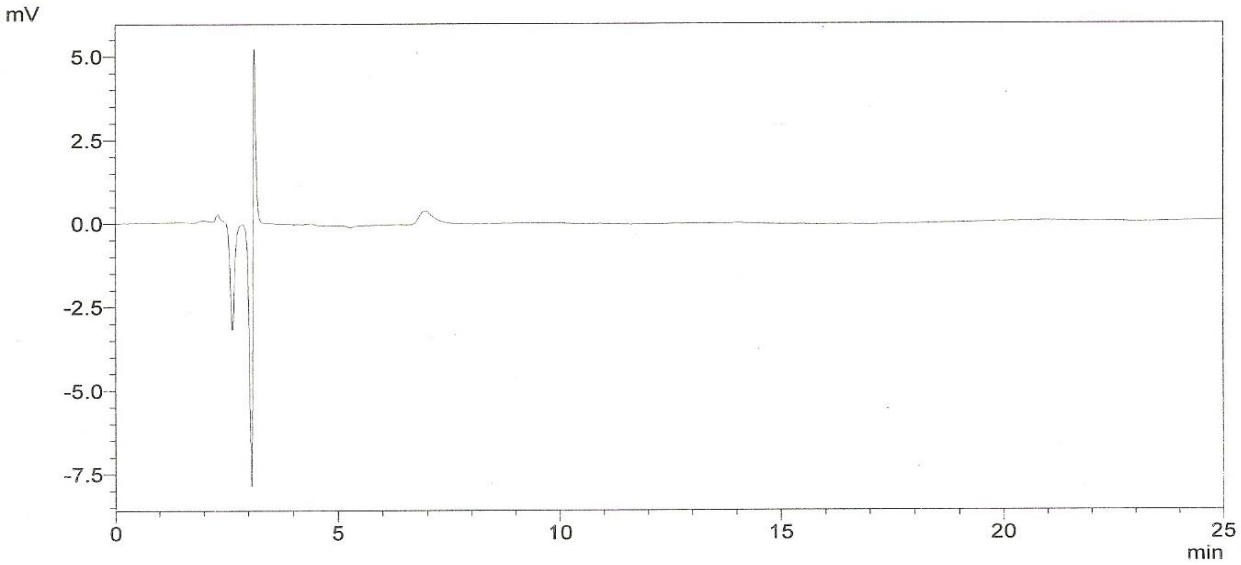

Fig. 2: Placebo chromatogram

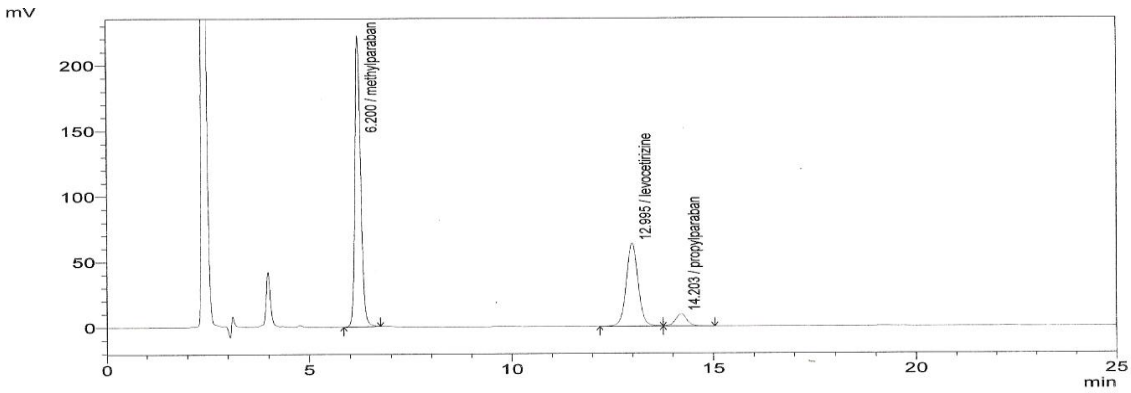

Fig. 3: Typical chromatogram of Levocetirizine, Methylparaben and Propyl paraben (standard)

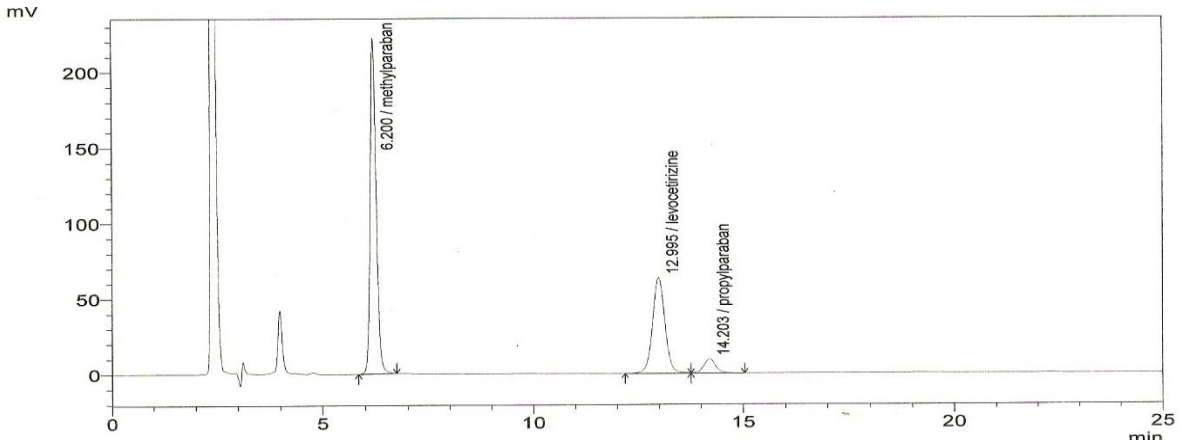

Fig. 4: Typical chromatogram of Levocetirizine, Methylparaben and Propylparaben (sample)

SPECIFICITY

Placebo Interference: A study to evaluate the interference of placebo was conducted. Samples were prepared by taking placebo equivalent to the weight present in portion of test preparation as per the test method and injected into the HPLC system. The Chromatogram indicates that the peak is homogeneous, there is no interference from the excipients at the retention time of analyte peak and has no co-eluting peaks indicating specificity of the method (Fig. 2 - 4).

Interference from Degradation products

A forced degradation study was conducted to demonstrate the stability and specificity. Separate portion of the drug product and placebo were exposed to following stress conditions to induce degradation.

1. Treated with HCl solution.
2. Treated with NaOH solution.
3. Treated with 3%Hydrogen peroxide.
4. Exposed to Humidity at 25°C, 90%RH for about 72 hours.
5. Exposed to Heat at about 105°C temperature for about 48 hours.
6. Exposed to UV light for about 24 hours.

Stressed samples were analyzed as per test method. The chromatograms of the stressed samples were evaluated for the peak purity. For all the forced degradation samples the peak purity was found to be within the limit. Data shown in Table 4 indicates peaks are homogeneous and that there are no co-eluting peaks indicating that the method is stability indicating and specific.

INTRFERENCE FROM THE DEGRADATION PRODUCTS

Degradation mechanism / condition	Observation
Acid degradation 0.1 N HCl Reflux – 30 min	No interference at RT of analyte peak
Base degradation 0.01 N NaOH Reflux - 45min	No interference at RT of analyte peak
Peroxide degradation 3% H_2O_2 Reflux – 30min	No interference at RT of analyte peak
Thermal degradation At 105°C - 48 Hrs	No interference at RT of analyte peak
Photolytic degradation At 254nm - 24 Hrs	No interference at RT of analyte peak
Water Reflux – 30 min	No interference at RT of analyte peak

Table 4: Forced Degradation Studies for method

Experiment	Condition	% Assay of Levocetirizine	% Degradation	Purity angle	Purity Threshold
Acid degradation	0.1 N HCl -Reflux 30 min	99.7	0.3	0.133	0.319
Alkali degradation	0.01 N NaOH -Reflux 45min	88.1	11.9	0.087	0.300
Peroxide degradation	3% H_2O_2 -Reflux 30min	94.8	5.2	0.087	0.299
Thermal degradation	At 105°C - 48 Hrs	68.4	31.6	0.247	0.287
Photolytic degradation	At 254nm - 24 Hrs	99.7	0.3	0.073	0.275
Water	Reflux – 30 min	98.4	1.6	0.081	0.291

LINEARITY

Linearity of detector response was established by plotting graph between concentrations and average area counts of the analytes. Data shown in Table 5, and represented graphically Fig 5-7 indicates that the response is linear over the specified range.

Table 5: Results of Linearity of Method

% Level	Levocetirizine		Methyl Paraben		Propyl Paraben	
	Concentration (µg/mL)	Average area counts(uv*sec)	Concentration (µg/mL)	Average area counts(uv*sec)	Concentration (µg/mL)	Average area counts(uv*sec)
L1-25%	12.57	1343950	17.1	71720	10	71720
L2-50%	25.15	2687900	34.2	141440	20.1	141440
L3-75%	37.72	4131872	51.3	212160	30.2	212160
L4-100%	50.3	5375837	68.4	282687	40.3	282687
L5-125%	62.87	6719787	85.5	353600	50.4	353600
L6-150%	75.45	8063745	102.6	415320	60.5	415320
Slope		106645	Slope	4052	Slope	6860
Intercept		27085.3	Intercept	3653.8	Intercept	4339.8
Correlation coefficient		0.9999	Correlation coefficient	0.9998		0.9998

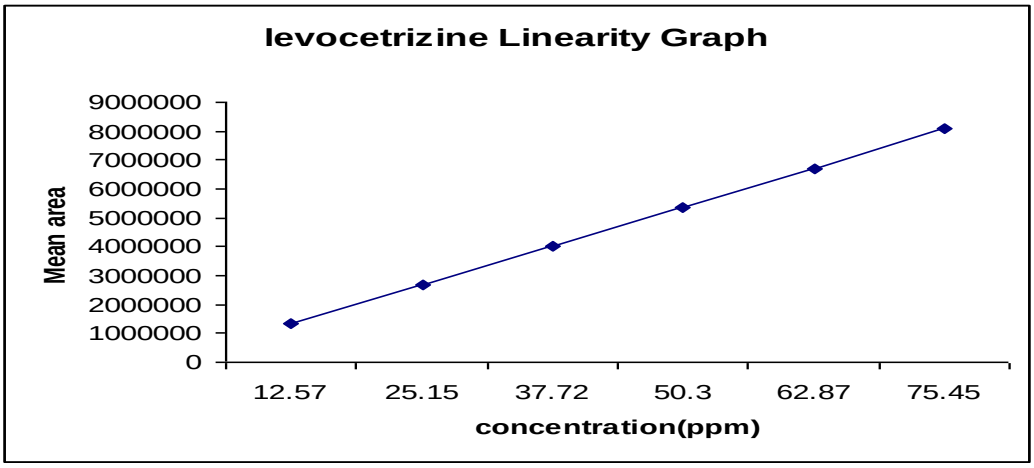

Fig. 5: Linearity graph of Levocetrizine

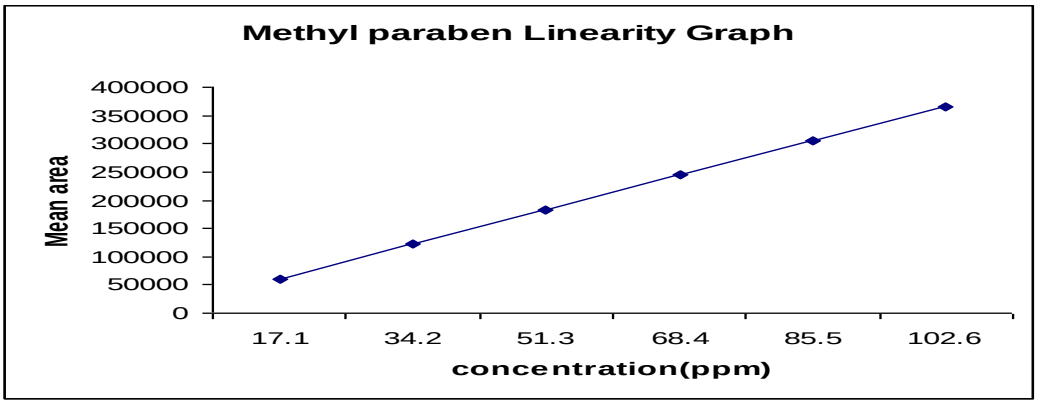

Fig. 6: Linearity graph of Methylparaben

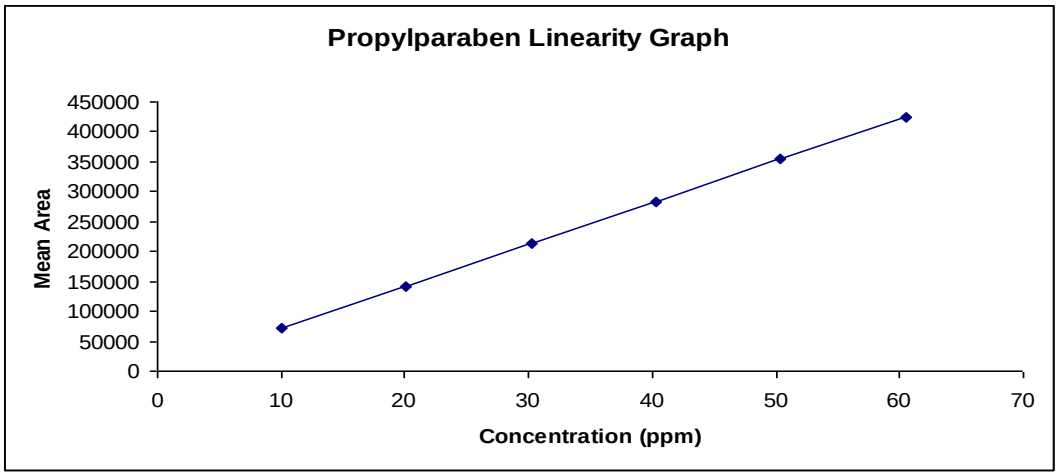

Fig. 7: Linearity graph of Propylparaben

ACCURACY

A study of accuracy (recovery) was performed on known amount of placebo by spiking API. Samples were prepared as per the proposed method at three levels in triplicate**.** Data shown in Table 6-8 for method indicate that the method has an acceptable level of accuracy.

STABILITY IN SAMPLE SOLUTION

A study to establish stability of sample solution was conducted on bench top. A sample solution was prepared as per proposed method, it was analysed initially and at different time intervals. Data shown in Table -9 as the difference in % assay meets the acceptance criteria.

Table 6: Results of Accuracy

Recovery Levels	Levocetirizine				
	Amount added (mg)	Amount recovered (mg)	%Recovered	Mean % recovery	% RSD
50% Rec-1	25.1	24.79	**98.8**		
50% Rec-2	25.05	24.46	**97.6**		
50% Rec-3	25.05	24.53	**97.9**	**98.1**	**0.59**
100% Rec-1	50.2	49.45	**98.5**		
100% Rec-2	50.1	49.45	**98.7**		
100% Rec-3	49.95	49.1	**98.3**	**98.5**	**0.21**
150% Rec-1	75.5	74.25	**98.3**		
150% Rec-2	75.53	74.1	**98.1**		
150% Rec-3	74.95	73.4	**97.9**	**98.1**	**0.21**

Table 7: Results of Accuracy

Recovery Levels	Methyl Paraben				
	Amount added (mg)	Amount recovered (mg)	%Recovered	Mean % recovery	% RSD
50% Rec-1	34.23	33.75	**98.6**		
50% Rec-2	34.15	33.39	**97.8**		
50% Rec-3	34.05	33.74	**99.1**	**98.5**	**0.67**
100% Rec-1	68.46	67.91	**99.2**		
100% Rec-2	68.32	67.35	**98.6**		
100% Rec-3	68.1	66.94	**98.3**	**98.7**	**0.47**
150% Rec-1	102.69	101.86	**99.2**		
150% Rec-2	102.45	101.52	**99.1**		
150% Rec-3	102.15	100.82	**98.7**	**99.0**	**0.26**

Table 8: Results of Accuracy

Recovery Levels	Propyl Paraben				
	Amount added (mg)	Amount recovered (mg)	%Recovered	Mean % recovery	% RSD
50% Rec-1	20.11	19.83	**98.6**		
50% Rec-2	20.02	19.57	**97.8**		
50% Rec-3	20.16	19.97	**99.1**	**98.5**	**0.67**
100% Rec-1	40.34	40.01	**99.2**		
100% Rec-2	40.27	39.7	**98.6**		
100% Rec-3	40.42	39.73	**98.3**	**98.7**	**0.46**
150% Rec-1	60.43	59.94	**99.2**		
150% Rec-2	60.34	59.79	**99.1**		
150% Rec-3	60.21	59.42	**98.7**	**99.0**	**0.27**

Table 9: Stability in sample solution

Drug name	Time	% Assay	Difference
Levocetirizine	Initial	99.9	1.3
	About 48hr	98.6	
Methyl Paraben	Initial	99.1	0.7
	About 48hr	98.4	
Propyl Paraben	Initial	99.8	1.2
	About 48hr	98.6	

CONCLUSION

A new stability- indicating RP-HPLC method has been developed for estimation of Levocetrizine dihydrogenchloride active pharmaceutical ingredient. The method was validated for accuracy, precision, specificity, and linearity. The forced degradation studies were carried out in accordance with ICH guidelines and the results revealed suitability of the method to study stability of Levocetirizine under various degradation conditions like acidic, basic, oxidative, thermal, UV and photolytic degradations. The drug solutions employed in the study were stable upto 48 hours. These attribute the high quality of the method. The proposed method can be used for the estimation of Levocetrizine dihydrogenchloride in bulk drug preparation and in pharmaceutical dosage forms for routine analysis in quality control laboratories. Finally it was concluded that the method is simple, sensitive and has the ability to separate the drug from degradation products found in pharmaceutical dosage forms.

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