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Research article

RP-HPLC method development for the estimation of Levocetirizine and Phenylephrine hydrochloride in combined dosage form

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ABSTRACT

A rapid, accurate RP-HPLC method was developed for the simultaneous estimation of Levocetirizine and phenylephrine hydrochloride in combined dosage form. The HPLC separation was achieved on column Luna 5u C18 (20mm X 4mm) using mobile phase of methanol: Pot. Dihydrogen Phosphate Buffer (pH 6.0), (70:30) .The UV detection was performed at 251nm with flow rate of 1.0 mL/min. The retention time of Levocetirizine and Phenylephrine hydrochloride was found to be 8.42 min and 2.70 min with mean recovery 100.64 & 100.40% respectively. The proposed method was found useful in the routinely quantification of both drugs in combined dosage form.

1. Introduction

Levocetirizine dihydrochloride is chemically dihydrochloride salt of (R)-2-(2-(4-((4-chlorophenyl) phenyl methyl) piperazin-1-yl) ethoxy) acetic acid[1] and its chemical structure is drawn in figure 1. It is used as antihistamine mediated via selective inhibition of H_1 receptors[2]. Levocetirizine dihydrochloride is available in number of combinations with montelukast, diethylcarbamazine, nimesulide, pseudoephedrine, cefpirome, while multi-component combination of gliquidone, fexofenadone, buclizine and phenylephrine hydrochloride, guaiphenesin, ambroxol hydrochloride with levocetirizine dihydrochloride is also available.

Phenylephrine hydrochloride is chemically hydrochloride salt of (R)-1-(3-hydroxyphenyl)-2methyl amino ethanol[3] and its chemical structure is drawn in figure 2. It is used as decongestant, cold by causes vasoconstriction of the arterioles of the nasal mucosa and conjunctiva; activates the dilator muscle of the pupil to cause contraction; produces vasoconstriction of arterioles in the body and produces systemic arterial vasoconstriction[4]. Phenylephrine hydrochloride is available in combination with ebastine, chlorpheniramine maleate, tropicamide. It is available in number of multi-component combination as acetaminophen, chlorpheniramine and guaifenesin, paracetamol and acetaminophen, phenylephrine and carbinoxamine.

A plethora of literature was reviewed for both drugs revealed a handful of method developments for individual drug estimation and or with the available multi-component combinations. These accounts the RP-HPLC, HPTLC, Spectro-flourimetric, LC-MS, Colorimetry, Direct spectrophotometry method developments while utilizing buffers, oxidizing agents, alkalis and number of other chemicals step by step[5-19]. Neither HPLC nor any other method development has been reported for the selected combination. Thus listed number of method developments provided a clue for the proposed research work for the simultaneous estimation of levocetirizine dihydrochloride and phenylephrine hydrochloride in combined dosage form. The present work endowed with an advantage of estimating a minimum concentration of each drug (Levocetirizine dihydrochloride & phenylephrine hydrochloride) at one time with accuracy.

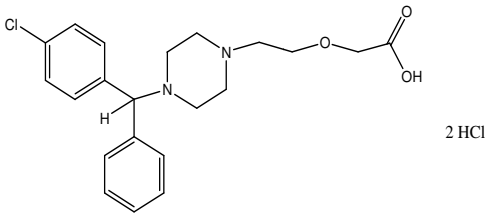

Figure.1 Chemical structure of levocetirizine dihydrochloride

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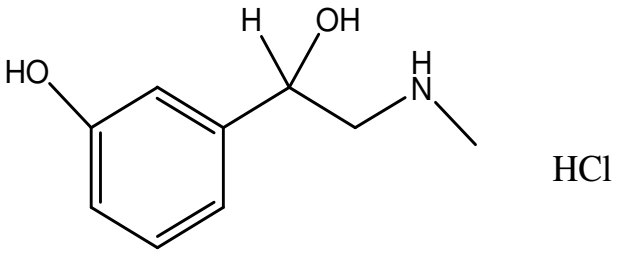

Fig.2 Chemical structure of Phenylephrine Hydrochloride

2. Experimental

2.1. Material

Levocetirizine dihydrochloride and Phenylephrine hydrochloride were obtained as generous gift sample from Swiss Garnier Pharmaceuticals Limited, Mehatpur (HP) India. FC-TAB (Hetero Healthcare, India) was purchased from local market. HPLC grade methanol, Water, Ethanol were procured from Merck Chemicals, Mumbai, India. Potassium dihydrogen phosphate, potassium hydroxide, disodium hydrogen phosphate of analytical grade were purchased from Merck Chemicals, Mumbai. The mobile phase and all the solutions were filtered through 0.45µm nylon membrane filter.

2.2. Instrumentation

2.2.1. High performance liquid chromatography

Shimadzu HPLC system equipped with prominence LC-20 AD pump, with SPD – 20 A prominence UV- Visible detector. The output signal was monitored and integrated by LC Solutions software for the analysis. The manual sampler was used with loop 20µl injection volume.

2.2.2. Chromatographic conditions

Luna 5u C_{18} column (20 x 4mm) was used as stationary phase for separation. The mobile phase consisted of Methanol: Pot.dihydrogen phosphate buffer (pH 6.0) (70:30) with optimized flow rate for analysis was 1ml/min covering run time of 10 mins. The variable wavelength UV/VIS detector was set at a wavelength of 251 nm. Column was operated at room temperature.

2.3. Preparation of stock solutions

2.3.1. Preparation of standard stock solutions of LEVC and PHE

Standard stock solution (1mg/ml) of LEVC and PHE were prepared by transferring 50mg of LEVC and PHE in 50 ml volumetric flasks separately, dissolved in diluent (methanol) with sonication for 10 minutes and diluted upto mark with diluent (methanol). Filtered these solutions through 0.45µm nylon membrane filter and filtrate were used for further dilutions. From the standard stock solutions, mixed standard solution was prepared containing 25µg/mL and 50µg/mL for LEVC and PHE respectively.

2.3.2. Preparation of sample stock solution of LEV and PHE

Twenty tablets (FC-tab) were weighed and powdered. Quantity of powder equivalent to 5mg LEVC,10 mg PHE were transferred to standard 100 ml volumetric flask. The content was extracted by 10 min sonication with HPLC grade methanol and volume was made up to mark. The solution was filtered with a 0.45µm nylon membrane filter. Aliquot filtrate was further diluted to achieve final concentration of linearity range and concentration of LEVC and PHE can be estimated by direct comparison method.

2.4. Development and optimization of HPLC method

With the optimized chromatographic conditions, a steady baseline was recorded. Preliminary trials were taken with listed solvents as methanol, disodium hydrogen phosphate buffer (pH 5.7); Potassium dihydrogen phosphate buffer (pH 6.5, 6.0) with different ratio systems to obtain good resolution of peak. A typical chromatogram for LEVC and PHE is shown in figure 3.

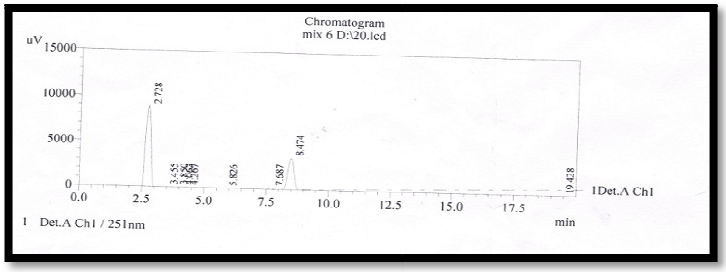

Fig. 3: Chromatogram of LEVC and PHE

2.5. Validation of developed method

Analytical method validation study involves testing multiple attributes of a method to determine that it can provide useful and valid data when used routinely. The method was validated for different parameters including system suitability, linearity, specificity, precision, accuracy (by recovery studies) and robustness according to the ICH guidelines (2005)[20].

2.5.1. System suitability

A system suitability testing is an integral part of analytical method. The system suitability test was performed collecting data from replicate injections of mixed drug solutions and peak area, Retention Time, theoretical plates and tailing factor were calculated for standard solutions.

2.5.2. Linearity

Linearity study was carried out with mixture of LEVC and PHE in five different concentration range of 30 -150% of the target

concentration. Each concentration was injected in triplicate and linearity curve was plotted between concentration and area response.

2.5.3. Specificity

The specificity of method was determined by checking the interference of placebo with LEVC and PHE which was eluted by checking the peak purity of LEVC and PHE.

2.5.4. Accuracy and precision

Intraday and Interday accuracy was performed by adding known amounts of LEVC and PHE to placebo preparation. The actual and measured concentrations were compared. %Recovery was evaluated at three different concentration level, three sets were prepared and injected in duplicate while for precision %RSD of assay was calculated.

2.5.5. Robustness

The robustness of the method was studied by deliberate changes in the flow rate (±0.1ml/min) at constant concentration. Results were calculated on the basis of variation that should be within ±1.0 %.

3. Result and discussion

The RP-HPLC assay method was selected on the basis of drug solubility as both LEVC and PHE are water soluble. The optimized chromatographic conditions were flow rate as 1ml/min, run time of 10 ± 0.5min, the wavelength as 251nm, mobile phase and its ratio as methanol : Pot. dihydrogen phosphate buffer (pH 6.0) in the best resolution ratio of 70:30.The validation results and evaluation of marketed formulation results are summarized in Table I & Table II respectively.

Table I: Summarized results of method validation for LEVC and PHE

Parameters	Acceptance criteria	Results obtained	
		LEVC	PHE
Retention time		8.45	2.74
Peak asymmetry	NMT 2	0.86	1.12
Theoretical plate	NLT 2000	6094	1811.11
Linearity	r^2 = 0.995- 1	0.9984	0.9983
Precision	% RSD NMT 2	0.83	0.41
Accuracy			
Intraday	% Recovery 98- 102%	100.41	100.73
Interday		100.87	100.07
Robustness	% deviation NMT ± 1	0.092	0.086

Table II: Evaluation results of marketed formulation

Drugs	Mean (%) ± SD	% RSD	% SEM
LEV	100.20 ± 0.035	0.701	0.020
PHE	99.70 ± 0.055	0.552	0.032

3.1. System suitability

The values obtained demonstrated the suitability of the system for the analysis as all its parameters fall within 2% coefficient of variation (%RSD) during routine performance of the method. The results are shown in table III.

Table III. System suitability results

Parameters	LEVC	PHE
Retention time	$\bar{X}$ = 8.55 ± 0.07 % RSD = 0.82 SEM = 0.0286	$\bar{X}$ = 2.74 ± 0.01 % RSD = 0.37 SEM = 0.0041
Peak area	$\bar{X}$ = 79093 ± 653.29 % RSD = 0.83 SEM = 266.7025	$\bar{X}$ = 137279 ± 771.27 % RSD = 0.56 SEM = 314.8697
Theoretical plates	$\bar{X}$ = 6094 ± 88.53 % RSD = 1.45 SEM = 36.1422	$\bar{X}$ = 1811.11 ± 12.61 % RSD = 0.70 SEM = 5.1480
Tailing factor	$\bar{X}$ = 2.13 ± 0.03 % RSD = 1.41 SEM = 0.0122	$\bar{X}$ = 1.12 ± 0.02 % RSD = 1.79 SEM = 0.0082

3.2. Linearity

Statistical analysis of data shows linear response of the drug with correlation coefficient of 0.9984 & 0.9983 for LEVC & PHE respectively. A typical linearity curve is depicted in figure 4.

3.3. Specificity

The excipients mixture of the tablet has shown no any specific peak at the RT of the analyte peak. This shows that the excipients do not interfere with the analyte peak. This demonstrates that the assay is specific for LEVC and PHE estimation.

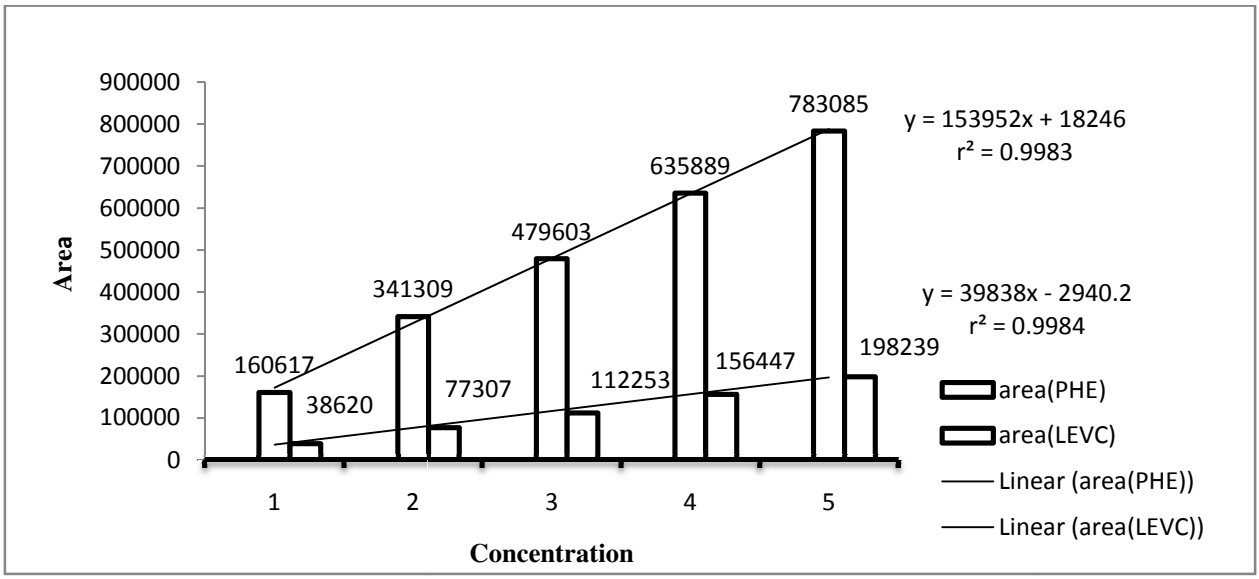

Figure 4: Linearity curve of mixture of LEVC and PHE

3.4. Accuracy and precision

The peak area responses for accuracy determination are depicted in Table IV. The results obtained shows average mean recovery of 100.64% and 100.40% for LEVC and PHE respectively indicating method was accurate while Intraday and Interday assay results were found to be precised as % RSD were 0.83% & 0.41% for LEVC and PHE respectively, thus confirms the method is precised.

Table IV: Results of accuracy studies by recovery

Drug	Parameters	Label claim	Amount added	Calculated conc. ± SD	RSD (%)	Recovery (%)
LEVC	*Intraday*	5 mg	80%	4.010 ± 0.030	0.748	100.25
			100%	4.993 ± 0.076	1.530	99.86
			120%	6.067 ± 0.078	1.280	101.12
	Interday		80%	4.020 ± 0.053	1.316	100.50
			100%	5.073 ± 0.045	0.889	101.05
			120%	6.063 ± 0.032	0.530	101.05
PHE	*Intraday*	10 mg	80%	8.103 ± 0.152	1.873	101.29
			100%	10.057 ± 0.065	0.647	100.57
			120%	12.040 ± 0.030	0.249	100.33
	Interday		80%	8.037 ± 0.031	0.380	100.46
			100%	9.990 ± 0.120	1.201	99.90
			120%	11.980 ± 0.155	1.296	99.84

3.5. Robustness

The results of robustness are depicted in Table III indicates that for the variance of flow 0.1ml/min, assay value of the test preparation solution was not affected and it was in accordance with that of actual. Thus the method proposed is robust.

Table V: Robust study results

Flow Rate (ml/min)	LEVC			PHE		
	Mean area ± SD	% SEM	% RSD	Mean area ± SD	% SEM	% RSD
0.9	7857667±45.004	25.983	0.057	1187368.67±113.006	65.244	0.060
1.0	78485.67± 72.05	41.603	0.092	187382.00 ± 160.614	92.731	0.086
1.1	78618.33±39.829	22.995	0.051	187413.33 ± 144.894	83.655	0.077

4. Conclusion

The developed HPLC method is confirmed to be novel, simple, precise, accurate, rugged and robust and is appropriate for its intended purpose. Validation makes this method suitable for its use in routinely quality control of simultaneous estimation of LEVC and PHE in marketed formulation. As there is no interference of any excipients in the analysis hence method is also specific with good resolution of peaks at run time of 10mins only.

5. Acknowledgement

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