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Development and Validation of RP-HPLC Method for Simultaneous Estimation of Levocetirizine Dihydrochloride and Phenylephrine in Bulk and In Tablet Dosage Form

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ABSTRACT

The present work deals with development and validation for simultaneous determination of antihistaminic drugs in pharmaceutical formulations. A rapid, precise and specific high performance liquid chromatography (RP-HPLC) method was developed for Levocetirizine dihydrochloride and Phenylephrine. Chromatographic separations was achieved on Waters Younglin system C-18 (5µm, 250×4.6 mm) HPLC column within a short runtime of 10 min. HPLC system having isocratic mode, with mobile phase containing methanol : water (pH 3) (70:30% *v/v*) and flow rate maintained at 1.0 mL/min was used. Effluents were monitored at 230 nm. Retention time of Levocetirizine dihydrochloride and Phenylephrine were found to be 2.6 and 4.6 min respectively. Linearity was studied in the concentration range of 2 to 12 µg/mL and 12 to 72 µg/ mL for Levocetirizine dihydrochloride and Phenylephrine respectively, with a correlation coefficient of 0.998 and 0.999 respectively. The proposed method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness.

Keywords: RP-HPLC, Levocetirizine dihydrochloride, Phenylephrine, Specificity, Validation

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INTRODUCTION

Levocetrizine (LEV) (Figure 1a), 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].

Chemically, Phenylephrine hydrochloride (PHE), is (*R*)-2-methylamino-1-(3-hydroxyphenyl) ethanol hydrochloride), it is an alpha-adrenergic (sympathomimetic) agent which stimulates alpha-adrenergic receptors, producing pronounced vasoconstriction[5]. Phenylephrine hydrochloride is official in IP [6], BP [7].

Several analytical methods have been reported for the analysis of LEV and PHE alone or in combination with other drugs such as few Spectrophotometric method[8, 11] chromatographic[12, 14]. Our objective in the present investigation is to develop and validate RP-HPLC method for simultaneous determination of Levocetrizine dihydrochloride (LEV) and Phenylephrine (PHE) and their analysis in tablets.

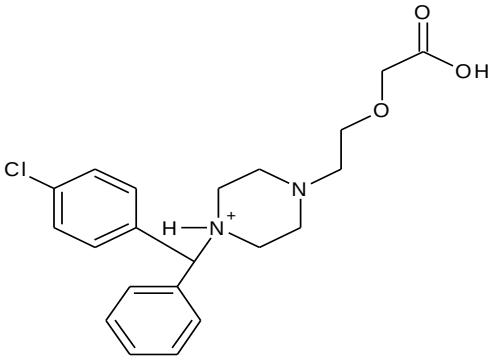

Figure 1: (a) Chemical structure of LEV

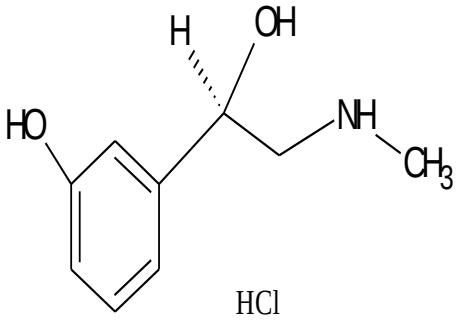

Figure 1: (b) Chemical structure of PHE

MATERIALS AND METHODS

Reagents and Samples

HPLC grade methanol and AR grade ortho-phosphoric acid were procured from Merck® India Ltd. (Mumbai). Pure standards of Levocetrizine dihydrochloride (99.26 %) and Phenylephrine (99.89 %) were obtained as gift samples from Glenmark Pharmaceutical Ltd. Nasik (India). Water was purified with Milli-Q Millipore system. All the solvents and solutions were filtered through a membrane filter (Millipore Millex® FH, filter units, Dura pore-PVDF, Polyethylene, 0.45 µm pore size) and degassed before use.

Instrumentation and Materials

Analysis was performed on Waters Younglin HPLC separation module within built UV-detector. Chromatographic software Empower 2 was used for data collection and processing. The analytical column was Phenomenex Gemini C 18 (5 µm, 250 mm X 4.6 mm).

Standard stock Solutions

Standard stock solutions were prepared by dissolving separately 10mg of LEV and PHE in 100 ml methanol (100µg/ml).

Preparation of tablet dosage form

Twenty tablets (Levocet) (each contained 5 mg LEV and 30 mg PHE) were accurately weighed and finely powdered. A quantity of the powder containing weight equivalent to 10 mg was transferred to a 100 mL volumetric flask and methanol (75 mL) was added followed by ultrasonication for 10 min. The solution was then diluted to volume with the same solvent and filtered. From that six solutions were prepared affording final concentrations of 10 µg/mL for LEV and 60 µg/mL for PHE and were used for further analysis.

VALIDATION

The proposed method was validated according to ICH (Q2) B guidelines for validation of analytical procedures. As per the ICH guidelines15 the method validation parameters checked were linearity, precision, accuracy, LOD and LOQ.

Linearity

For constructing calibration plots, a series of six dilutions in the concentration range 2-12 (2,4,6,8,10,12) µg/mL for LEV and 12-72 (12,24,36,48,60,72) µg/mL of PHE was taken. Linearity curve for LEV and PHE shown in Fig.3 & Fig.4 respectively.

Precision

In order to validate and prove the applicability of the method, a laboratory mixture of LEV and PHE was prepared from the stock solutions in the ratio corresponding to amounts in the dosage form. For quantitative estimation of the mixture, three series (4, 6, 8 µg/mL and 24, 36, 48 µg/mL for LEV and PHE respectively) were prepared, with three solutions for each concentration.

Recovery

The recovery experiments were performed by adding known amounts to tablet. The recovery was performed at three levels, 80, 100 and 120% of LEV and PHE standard concentration. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated by using formula.

$$\% \text{ Recovery} = \frac{\text{Observed amount of compound in Sample}}{\text{Amount of all compound present in Sample}} \times 100$$

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

The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × N/B and LOQ = 10 × N/B where 'N' is the standard deviation of the peak areas of the drug (n=3), taken as the measure of the noise, and 'B' is the slope of the corresponding calibration plot. The signal to noise ratio was determined. The LOD was regarded as the amount for which the signal to noise ratio was 3:1 and LOQ regarded as the amount for which the signal to noise ratio was 10:1**.**

Robustness

By introducing small but deliberate changes in the mobile phase composition (± 5.0%), detection and flow rate (± 0.9 - 1.1mL/min) robustness of the described method was studied.

RESULTS AND DISCUSSION

A RP-HPLC method was optimized with a view to develop an accurate and reproducible method so as to resolve drugs. Isocratic elution is simple, requires only one pump and flat baseline separation for easy and reproducible results. Optimization of method was done by altering mobile phase composition; pH, column packing, flow rate, temperature, detection wavelength and the effect on retention and peak shape were monitored for LEV and PHE. Typical chromatogram of LEV and PHE shown in figure.2.

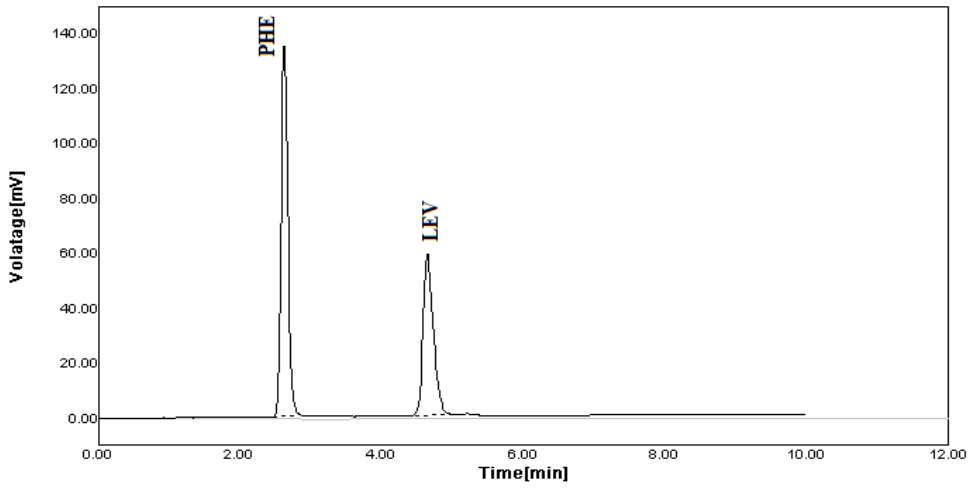

Figure 2: Typical chromatogram of LEV and PHE.

The method showed good linear response in concentration range of 2-12 µg/mL for LEV (r^2 =0.998) and 12-72 µg/mL for PHE (r^2 =0.999) (Table 1). The method was found to be precise and RSD was found to be less than 2.0% (Table 2). The recovery values were 99.11-101.09% foe LEV and 99.77-100.12 for PHE with R.S.D. of < 2 (Table 3).Assay of LEV and PHE shown in

Table 4.LOD and LOQ value of LEV and PHE in Table 5.Table 6 shows robustness of the method. The final chromatographic conditions were shown in Table 7.

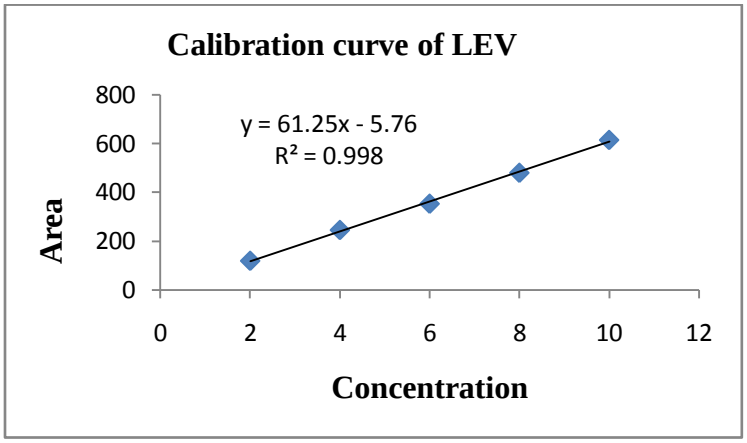

Figure3: Linearity curve for LEV

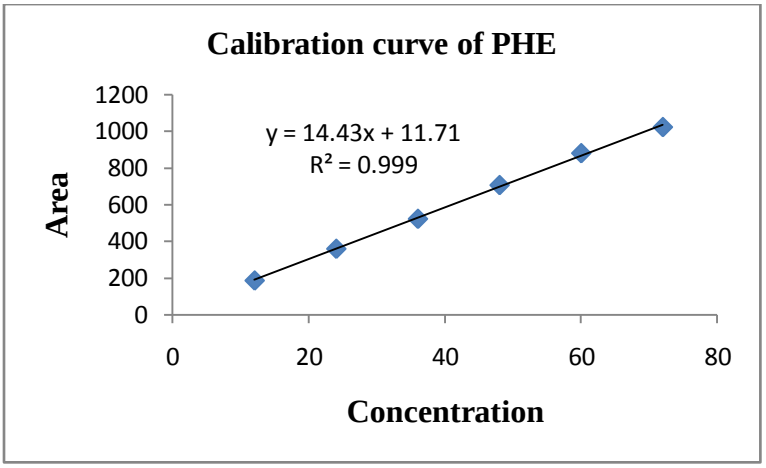

Figure4: Linearity curve for PHE

Table 1: Linearity of LEV and PHE for proposed method (n=6)

Parameters	LEV	PHE
Linear range (µg mL^{-1})	2 – 12	12 – 72
Slope	61.25	14.43
Intercept	5.76	11.71
Correlation Coefficient (r^2)	0.998	0.999

Table 2: Precision of the RP–HPLC method

Drugs	Conc. [µg/mL]	Intraday Amount found [µg]		Interday Amount found [µg]	
		Mean ± S.D.	% R.S.D*	Mean ± S.D.	% R.S.D*
LEV	4	4.04 ± 3.8	1.60	4.03 ± 3.5	1.47
	6	5.91 ± 4.4	1.24	5.87 ± 6.0	1.69
	8	7.90 ± 4.1	0.86	7.92 ± 5.6	1.17
PHE	24	24.15 ± 5.8	1.62	24.26 ± 5.4	1.49
	36	35.37 ± 6.0	1.15	35.46 ± 7.5	1.43
	48	48.14 ± 11.7	1.66	48.18 ± 7.0	0.99

*mean of three determinations

Table 3: Recovery studies

Drugs	Initial Amount [µg/mL]	Amount added [µg/mL]	Amount recovered* ± S.D. [µg/mL]	%Recovered	%R.S.D.
LEV	5	4	8.92± 7.02	99.11	1.29
	5	5	10.05 ± 6.02	100.5	0.98
	5	6	11.12 ± 6.03	101.09	0.89
PHE	30	24	54.07 ± 6.55	100.12	0.82
	30	30	60.19 ± 6.50	100.31	0.73
	30	36	65.85 ± 6.50	99.77	0.67

*mean of three determinations

Table 4: Tablet Assay

Drugs	Label claim [mg/Tab]	% label claim*	% R.S.D.
LEV	5.0	100.76	1.07
PHE	30.0	101.70	1.02

*mean of six determinations

Table 5: LOD and LOQ of the method

Drugs	LOD	LOQ
LEV	0.19	0.57
PHE	2.9	8.71

Table 6: Robustness of the method

Conc. [µg/ml]	Retention time (R_T)		Tailing factor (Tf)	
	LEV	PHE	LEV	PHE
A: Flow Rate (ml/min)				
0.90	4.9	3.1	0.89	1.16
1.00	4.6	2.6	1.40	1.22
1.10	3.6	2.0	1.54	1.11
Mean	3.46	7.91	1.22	1.1
B: Percentage methanol in mobile phase (v/v) (± 5%)	5.1	3.2	1.57	1.54
	4.6	2.6	1.36	1.31
	3.1	2.1	1.54	1.52
Mean	4.26	2.63	1.49	1.45

Table 7: Final Chromatographic Conditions

Chromatographic Mode	Chromatographic Condition
Standard solution (µg/mL)	1000 µg/mL of PHE and LEV in methanol
HPLC System	Younglin 1100 Series HPLC system
Pump	Gradient pump
Detector	UV Detector
Degasser	G1322A
Data processor	Ezechrome Elite Chromatographic data system
Weighing Balance	Shimandzu AUX 120
Digital pH Meter	Systronics µ pH System 362
Ultrasonicator	ENERTECH Electronics Pvt. Ltd.

Filters	Millipore (0.45 µm)
Stationary phase	Phenomenex Gemini C18 (5 µm, 250 mm X 4.6 mm i.d.)
Mobile phase	methanol: water (pH 3) (70:30% *v/v*)
Detection wavelength	230 nm
Flow rate	1 mL/min
Sample size	20 µL

CONCLUSION

A validated RP-HPLC method has been developed for the determination of LEV & PHE in bulk and in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of LEV and PHE in tablet formulation.

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