International Journal of Research in Pharmaceutical sciences and Technology

Simultaneous determination of levocetrizine and phenylpropanolamine hydrocholride by RP-HPLC

A. Srinivasan*, P. Divakar, A. Maheswaran, D. Saravanan

Department of Pharmaceutical Analysis, Jaya College of Paramedical Sciences, College of Pharmacy, Chennai-602024, Tamil Nadu, India.

ABSTRACT

The aim of the present study was to develop the simple, selective, rapid, precise and economical reverse phase-high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of levocetirizine and phenylpropanolamine HCl in solid dosage forms. The method was carried out on a Phenomenex Luna C18 (25 cm × 4.6 mm i.d., 5 µ) column with a mobile phase consisting of acetonitrile: 0.5% triethylamine (70:30 v/v, pH 3.0) at a flow rate of 1.2 mL/min. Detection was carried out at 220 nm. The retention time (RT) 1.8 min and 2.6 min for phenylpropanolamine hydrocholride and levocetrizine respectively. The % recovery of standard phenylpropanolamine hydrocholride and levocetrizine was found to be 98.17 to 103.56 and 98.893 to 10.422 respectively. The % recovery of sample phenylpropanolamine hydrocholride and levocetrizine was found to be 101.30 and 100.63 respectively. The validation of the proposed method was also carried out. The proposed method can be used for the estimation of these drugs in combined dosage forms.

Keywords: Phenylpropanolamine hydrochloride; Levocetrizine; Estimation; Validation; HPLC.

ISSN: 2581-9143
Research Article

Corresponding Author

Name: A. Srinivasan
Email: sriniarumugam92@gmail.com

Article Info

Received on: 14-09-2020
Revised on: 06-10-2020
Accepted on: 21-10-2020

DOI: https://doi.org/10.33974/ijrpst.v2i2.238

Copyright© 2020, A. Srinivasan, Simultaneous determination of levocetrizine and phenylpropanolamine hydrocholride by RP-HPLC, Production and hosting by *Rubatosis Publications*.

INTRODUCTION

Levocetirizine (LEVO) (Figure 1) is a non-sedative antihistamine (third generation) that are developed from cetirizine (second-generation antihistamine). LEVO is active enantiomer of cetirizine which is chemically 2-(2-(4-((R)-(4-chlorophenyl)-phenyl-methyl) piperazin-1-yl) ethoxy) acetic acid dihydrochloride. LEVO is a highly effective drug with fewer side effects when compared to second generation drug, cetirizine. [1,2]

Phenylpropanolamine (PPH) is benzemethanol, α-(1-aminoethyl)-hydrochloride, (R^*,S^*)-, (±).(±) Norephedrine hydrochloride (Figure 2) which is a mainly indirect acting sympathomimetic drug with an action similar to ephedrine. It is orally administered for the treatment of nasal congestion, relief of cough, cold symptoms, control of the urinary incontinence in some patients and suppress appetite in the management of obesity.[3]

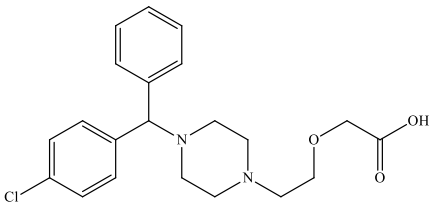

Figure 1: Structure of levocetirizine

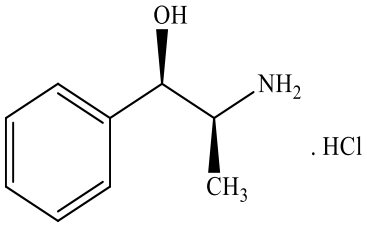

Figure 2: Structure of phenylpropanolamine HCl

Literature survey reveals that the a number of analytical methods have been reported for the estimation of LEVO and PPH as individual and combined dosage form with each other and with other combination of other drugs such as Spectrophotometry, potentiometry, capillary gas chromatography and HPLC method. [4-12] The aim of present work was to develop simple, sensitive, accurate, and precise methods for the simultaneous estimation of levocetirizine and phenylpropanolamine HCl in solid dosage forms for

routine analysis. The proposed method was validated according to ICH guidelines. [13]

MATERIAL AND METHODS

Apparatus and software: The Shimadzu HPLC system consisting Binary gradient pump (LC-20AD VP pump), mixer (SUS vp Assy (new), Rheodyne injector, PDA Multiple wavelength detector SPD - M20A vp) and Hamilton syringe (all from Shimadzu, Kyoto Japan) was used. The separations were achieved on a Phenomenex Luna 5µ C18 (2) 100A, 250X4.60mm, with UV detection at 220 nm. Analytical weighing balance (Shimadzu AUX 200) was used for weighing, sonicator (SONICA 2200MH), vacuum pump (model XI 5522050 of Millipore), Millipore filtration kit for solvents and sample filtration were used throughout the experiment. The LC solution software-multiple channel was used for acquisition, evaluation and storage of chromatographic data.

Reagents and Pharmaceutical Preparations: LEVO and PPH were kindly gifted by Mepro Pharmaceuticals Ltd, Gujrat and Embiotic Ltd, Bangalore, Certified to contain 99.86% and 99.92% purity respectively. The drugs are used without further purification. All the solvents used in analysis were of HPLC grade. Lezyncet-D tablets (label claim 5mg LEVO and 25mg PPH).

Standard solutions of LEVO and PPH

It was used stock solutions of LEVO and PPH 1mgmL^{-1} in Acetonitrile: 0.5% Triethylamine (70:30 v/v, pH 3.0). The working solutions were 0.08 mg mL^{-1} and 0.10mg mL^{-1} prepared by transferring 2.0 mL and 4.0 mL from respective stock solution to a 50 mL volumetric flask and completing to volume with mobile phase.

Preparation of mobile phase: HPLC experiments were carried out using Binary pump. In one solvent reservoir Acetonitrile and in another 0.5% Triethylamine (70:30 v/v, pH 3.0)

Pharmaceutical Sample solution: Lezyncet-D tablets were weighed accurately. An amount of the powder equivalent to content of one unit of tablet was dissolved separately in 60 ml of mobile phase. The solutions were sonicated for 10 min and filtered into a 100 ml volumetric flask through 0.45µ nylon membrane filter. The residue was washed 3 times with 10 ml of mobile phase, and then the volume was completed to 100 ml with the same solvent. These solutions were further diluted to 1:100 with mobile phase. The proposed RP-HPLC method was applied and the concentration of each component in the formulation was determined.

RESULTS AND DISCUSSIONS

Chromatography: The mobile phase in Acetonitrile: 0.5% Triethylamine (70:30 v/v, P^{H} 3.0) was selected, because it was found that it ideally resolve the peaks with retention time (RT) 1.8 min and 2.6 min for PPH and LEVO respectively and the same is shown in (Figure 3). Wavelength was selected by scanning both standard drugs over a wide range of wavelength 200nm to 350nm. Both the components show reasonably good response at 220 nm.

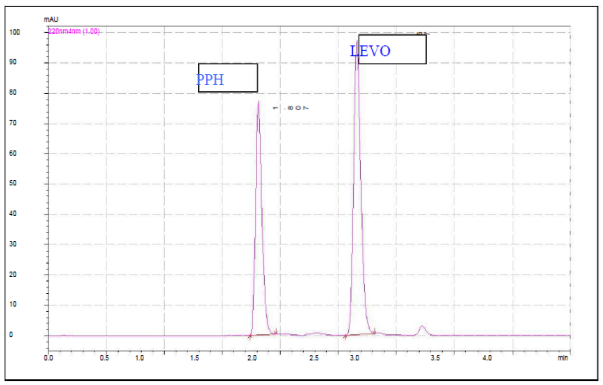

Figure 3: Chromatogram of LEVO and PPH

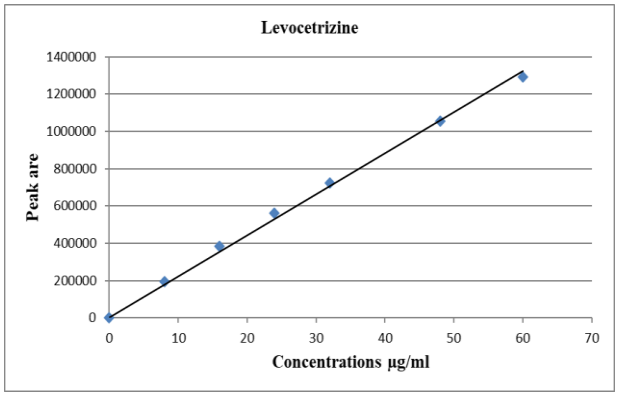

Figure 4: Calibration curve of levocetrizine

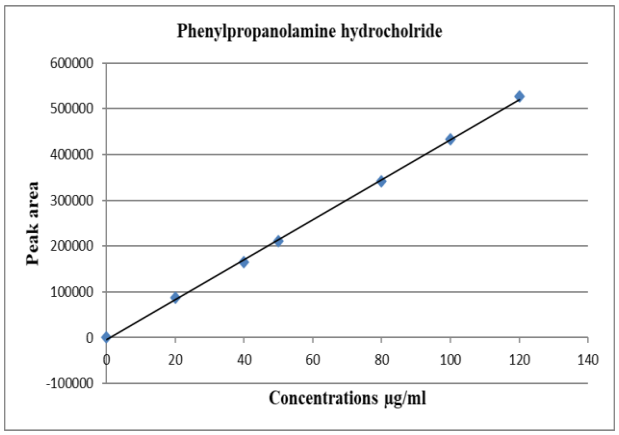

Figure 5: Calibration curve of Phenylpropanolamine hydrocholride

Validation of HPLC method

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application.

To check the validity (predictive ability) of the calibration models, the simultaneous analysis of the prediction set containing 16 samples of various concentrations (in triplicates) of LEVO and PPH was

Table 1: Composition of the concentrations and recoveries results

S. No	Concentration in µg/ml		% Recovery		% Error	
	LEVO	PPH	LEVO	PPH	LEVO	PPH
1	8	20	99.05	104.15	-0.95	4.15
2	8	40	100.52	99.89	0.52	-0.11
3	8	80	101.43	99.82	1.43	-0.18
4	8	120	100.54	99. 76	0.54	-0.24
5	16	20	102.74	102.35	2.74	2.35
6	16	40	103.10	103.33	3.10	3.33
7	16	80	101.01	99.68	1. 01	-0.32
8	16	120	100.57	101.15	0.57	1.15
9	24	20	99.62	98.89	-0.38	-1.11
10	24	40	104.65	99.92	4.65	-0.08
11	24	80	102.64	101.00	2.64	-1.00
12	24	120	101.19	102.61	1.19	2.61
13	44	20	100.09	101.35	0.09	1.35
14	44	40	99.75	99.15	-0.25	0.85
15	44	80	102.05	101.06	2.05	1.06
16	44	120	101.64	98.94	1.47	1.06
Mean recovery value			101.27	100.81	1.29	0.929
%RSD			1.45	1.61		

Table 2: Linearity data for LEVO and PPH

S. No	Concentration of LEVO (g mL^{-1})	Peak area	Concentration of PPH (g mL^{-1})	Peak area
1	8	196001	20	87946
2	16	383838	40	164709
3	24	563397	50	210268
4	32	721833	80	341086
5	48	1053307	100	433252
6	60	1291337	120	526906

Table 3: Characteristic parameters of calibration equation for the proposed HPLC method for simultaneous determination of LEVO and PPH

S.no	Parameters	LEVO	PPH
1	Correlation coefficient	0.999	0.999
2	Calibration range (µg mL^{-1})	8 – 60	20-120
3	Detection limit (µg mL^{-1})	0.00028	0.0012
4	Quantitation limit (µg mL^{-1})	0.00086	0.0038
5	Regression equation (Y)a	0.997	0.9968
6	Slope (b)	16967	4248.1
7	Standard deviation of the slope (Sb)	16591	4147.3
8	Intercept (a)	54754	743.44

Table 4: Precision study results of prepared binary mixture

Validation parameter	HPLC % RSD		
Repeatability[a]			
LEVO	0.4011	0.93193	0.07646
PPH	0.4739	0.26356	0.04844
Intermediate precision[b]	**Peak area**	**Peak asymmetry**	**Retention time**
LEVO	1.2549	0.75072	0.03893
PPH	1.1821	0.39508	0.02174

[a]Repeatability, three replicates of four concentration levels within-day

[b]Intermediate precision, three replicates of four concentration levels between-days (3-days)

Table 5: Application of standard addition technique to the analysis of LEVO and PPH in Lezyncet-D tablets

S. No	Concentration of LEVO in µg/ml		% recovery ± SD	Concentration of PPH in µg/ml		% recovery ± SD
	Claimed	Added		Claimed	Added	
1	5	0	100.63 ± 0.104	25	0	101.30 ± 0.19
2	5	2	101.422 ± 0.050	25	4	98.17±0.150
3	5	4	98.893 ± 0.315	25	8	103.56±0.197

carried out and the mean recoveries and relative standard deviations of our proposed HPLC method was computed and indicated in (Table 1). Their numerical values were completely acceptable because of good recovery values and hence found satisfactory for the validation.

Linearity

The linearity of the proposed HPLC method for determination of LEVO and PPH was evaluated by analysing a series of different concentrations of standard drug. In this study six concentrations were chosen, ranging between 8-60 µg mL^{-1} of LEVO and 20-120 µg mL^{-1} of PPH. Each concentration was repeated three times and obtained information on the variation in the peak area response ratio of the internal standard to pure analytes is presented in (Table 2, Table 3). Area is plotted graphically as a function of analyte concentration (Figure 4, Figure 5). The linearity of the calibration graphs was validated by the high value of correlation coefficient, slope and the intercept value. A linear relationship was obtained for LEVO and PPH in the range of 8-60 g mL^{-1} and 20-120 g mL^{-1} respectively.

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

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to ICH (60) recommendations where the approach based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes was compared with the signals of blank samples. A signal-to-noise ratio 3:1 and 10:1 is considered for calculating LOD and LOQ respectively. The values of LOD and LOQ are given in (Table 5).

Precision

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision expressed as % RSD is given in (Table 3). Method reproducibility was demonstrated by repeatability and intermediate precision measurements of peak area, retention time and peak symmetry parameters of HPLC method for each title ingredients. The repeatability (within-day in triplicates) and intermediate precision (for 3 days) was carried out at four concentration levels for each compound. The obtained results within and between days trials are in acceptable range indicating good precision of the proposed methods (Table 4).

Accuracy

The study was performed by increasing standard addition of known amounts of studied drugs to an unknown concentration (constant volume) of the commercial pharmaceutical formulations (Standard addition access the effect of a sample matrix changes the analytical sensitivity of the method). A constant volume of the unknown solution is added to each of three 10 mL volumetric flasks. Then a series of increasing volumes of working standard solutions are added. Finally, each flask is made up to the mark with solvent and mixed well. The concentration of the working standard solutions added should be chosen to increase the concentration of the unknown by minimum 30% in each succeeding flask. The resulting mixtures were analysed by the proposed HPLC methods and the response obtained was plotted against the initial unknown concentration set at 0. The results obtained are compared with expected results. The excellent mean recoveries and standard deviation (Table 5) suggested good accuracy of the propose methods and no interference from formulations recipients.

Analysis of commercial formulations

Applicability of the method was tested by analyzing the commercially available formulations containing the binary mixture of LEVO and PPH. The values of % recovery from formulation as shown in the (Table 6) are found to be very close to each other as well as to the label value of commercial pharmaceutical formulation, shows that the method is applicable for simultaneous determination of LEVO and PPH from their binary mixture formulation.

(Lezyncet-D tablets label claim: 5 mg of LEVO and 25 mg PPH per tablet), [a]Mean recovery value of five determinations. [b]Standard deviation.

Table 6: Results obtained for the pharmaceutical samples by using formulation

Methods	Concentration in µg mL^{-1}	% recovery mean[a] ± SD[b]
LEVO	5	100.63 ± 0.104
PPH	25	101.30 ± 0.19

CONCLUSION

Proposed study describes a new RP-HPLC method for the simultaneous determination of LEVO and PPH. For routine analytical purpose it is desirable to establish method capable of analyzing large numbers of samples in a short time period with good accuracy and precision without any prior separation step. The

developed method gives good resolution between both the compounds with a short analysis time (<4 min). The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of LEVO and PPH in their combined dosage form.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

ACKNOWLEDGEMENT

Authors are thankful to Management and Principal, Jaya College of Paramedical Sciences, College of Pharmacy, Chennai, Tamil Nadu, India for providing all the facilities to carry out the work.

REFERENCES

1. Sahu, S.K., Azam, A., & Banarjee, M. (2011). Spectrophotometric Estimation of Moxifloxacin in Bulk and its Pharmaceutical Formulations. Pharmacologyonline, 2, 491-502.
2. Dinesh, M.D., Atul, A.S., Sanjay, J.S. (2011). Quantitative determination of Moxifloxacin hydrochloride in bulk and ophthalmic solution by UV-spectrophotometry and first order derivative using area under curve. Scholars Research Library Der Pharmacia Lettre, 3(3), 453-456.
3. Najma, S., Mahwish, A., Sana, S., Somia, G., Muhammad, S.A., Moona, M.K. (2010). HPLC assay for moxifloxacin in bulk and pharmaceutical formulations and serum application to invitro metal interactions. Journal of Chinese Chemical Society, 57 (4A), 2010.
4. Xu, Y.H., Li, D., Liu, X.Y., Li, Y.Z., Li, J. (2010). HPLC assay with ultraviolet detection for Moxifloxacin; validation and application to a pharmacokinetic study in Chinese volunteers. Journal of Chromatoraphy B, 878(32), 3437-3441. https://doi.org/10.1016/j.jchromb.2010.10.024
5. Dhillon, V., & Chaudhary, A. K. (2010). A validated HPTLC method for estimation of moxifloxacin hydrochloride in tablets. Pharmaceutical methods, 1(1), 54–56. https://doi.org/10.4103/2229-4708.72232
6. Chaudhary, R.S., Gangwal, S.S., Jindal, K.C., Khanna, S. (1993). Reversed-phase high performance liquid chromatography of ketorolac and its applicationto bioequivalent studies in human serum. Journal of Chromatography B: Biomedical Sciences and Applications, 180–184. https://doi.org/10.1016/0378-4347(93)80240-5
7. Sunil, G., Jambulingam, M., Ananda Thangadurai, S., Kamalakannan, D., Sundaraganapathy, R., Jothimanivannan, C. (2017). Development and validation of Ketorolac Tromethamine in eye drop formulation by RP-HPLC method. Arabian Journal of Chemistry, 10(1), S928-S935. https://doi.org/10.1016/j.arabjc.2012.12.031
8. Wu, A. T., & Massey, I. J. (1990). Simultaneous determination of ketorolac and its hydroxylated metabolite in plasma by high-performance liquid chromatography. Journal of chromatography, 534, 241–246. https://doi.org/10.1016/s0378-4347(00)82169-6
9. Ghandi, L.R., Dewani, A.P., Bakal, R.L., Chandewar, A.V. (2011). Absorption ratio method for the estimation of moxifloxacin hcl & ketorolac tromethamine in their combined dosage form by uv-visible spectroscopy. International Journal Pharmaceutical Medicine, 3(7), 21- 26.
10. Raju, B., Ramesh, M., Borkar, R. M., Padiya, R., Banerjee, S. K., & Srinivas, R. (2012). Development and validation of liquid chromatography-mass spectrometric method for simultaneous determination of moxifloxacin and ketorolac in rat plasma: application to pharmacokinetic study. Biomedical chromatography : BMC, 26(11), 1341–1347. https://doi.org/10.1002/bmc.2701
11. Razzaq, Syed Naeem, Khan, Islam Ullah, Ashfaq, Muhammad, & Mariam, Irfana. (2012). Stability indicating HPLC method for simultaneous determination of moxifloxacin hydrochloride and ketorolac tromethamine in pharmaceutical formulations. Química Nova, 35(6), 1216-1221. https://doi.org/10.1590/S0100-40422012000600028
12. Patel, D., Patel, M., Patel, K. (2012). Simultaneous RP-HPLC Estimation of Moxifloxacin Hydrochloride and Ketorolac Tromethamine in Ophthalmic Dosage Forms. Asian Journal of Research In Chemistry, 697.
13. Begum, S., Bharathi, K., Vaddepally, L., Rani, G. (2014). A validated RP-HPLC method for simultaneous estimation of moxifloxacin hydrochloride and ketorolac tromethamine in ophthalmic dosage form. Der Pharmacia Lettre, 6. 335-341.