International Journal of Pharmacognosy and Chemistry

Research Article

HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF LEVOCETIRIZINE AND PHENYLEPHRINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

P. Sreenivasa Prasanna , K.Thejomoorthy, P. Durga Bhavani*.

Department of Pharmaceutical analysis, M.L.College of Pharmacy, S.Konda-523101.

Abstract

A simple and precise and accurate analytical method was developed for The Simultaneous Estimation Of Levocetirizine And Phenylephrine In Bulk And Pharmaceutical Dosage Form, the analytical columm Eclipse Plus C18, 4.6 mm × 150 mm, (5 µm) ,Mobile phase consisting of Methanol: Water (50:50 iv/v) was used in isocratic mode. The mobile phase was initially filtered through 0.45µm millipore membrane filter and sonicated for 15 min before use. The flow rate was maintained at 1 mL/min, and the injection volume was 20µL. UV detection was performed at 277 nm, and the separation was achieved at ambient temperature.The retention time Rt in (min)for Levocetirizine and Phenylephrine 3.37 min and 6.40 min respectively . The linearity of the method was determined in the concentration range of 5-25µg/mL for Levocetirizine and 2.5-12.5µg/mL for Phenylephrine.

Keywords: Levocetrizine And Phenylephrine, Flowate, Wave Length, Retention Time.

Article Info

Received: 28-04-2020

Revised: 20-06-2020

Acepted: 23-06-2020

***Corresponding Author**

P. Durga Bhavani

Email: mlcollegeofpharamcy@gmail.com

INTRODUCTION

Drug analysis reveals identification characterization & determination of the drugs in mixtures like dosage forms & biological fluids. The number of drugs introduced in to the market has been increasing at high-speed rate. These drugs may be ether new entities in the Market or partial structural modification of the existing drugs (B.K. Sharma 2004). Newer analytical methods are developed for these drugs or drug combination of the below reasons: -

1. Official pharmacopoeia may not reveal an analytical procedure for the drugs or its combination.
2. The analytical method may not be available for the drug combination due to interference caused by excipients.

Levocetirizine is an antihistamine used for the treatment of allergic rhinitis (hay fever) and long term hives of unclear cause [1]. it is chemically called as 2-[2-[4-[(R)-(4-chlorophenyl)-phenyl methyl] piperazin-1-yl]ethoxy]acetic acid. It is less sedating than older antihistamines [2]. It is taken by mouth. Common side effects include sleepiness, dry mouth, cough, vomiting, and diarrhea [1]. Use in pregnancy appears safe but has not been well studied and use when breastfeeding is of unclear safety [3]. It is classified as a second-generation antihistamine and works by blocking histamine H1-receptors [4].

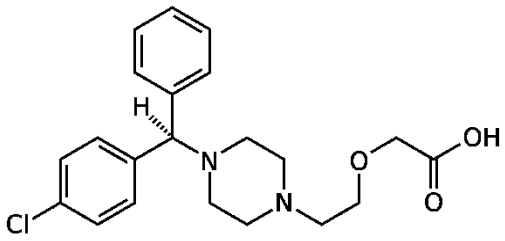

Figure 01: Chemical structure of Levocetirizine

Phenylephrine is used to relieve nasal discomfort caused by colds, allergies, and hay fever. It is also used to relieve sinus congestion and pressure. Phenylephrine will relieve symptoms but will not treat the cause of the symptoms or speed recovery. Phenylephrine is in a class of medications called nasal decongestants. It works by reducing swelling of the blood vessels in the nasal passages. It's chemically called as (−)-m-hydroxy-α-(methylaminomethyl) benzyl alcohol.

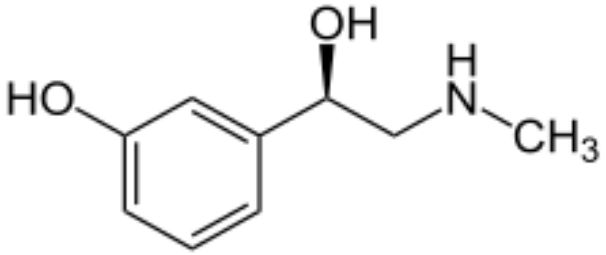

Figure 02: Chemical structure of Phenylephrine

After the literature survey, it was proved that there were few HPLC methods reported for the estimation of selected drugs of interest [5-6]. So in the present investigation we tried to establish a novel, stable and sensitive chromatographic method for the estimation of selected drugs.

EXPERIMENTAL WORK [7-15]

MATERIALS AND METHODS

Levocetirizine was procured from the Gift sample procured from Aurobindo Pharma Ltd., Hyderabad and Phenylephrine were procured Gift sample procured from Matrix Laboratories Ltd., Hyderabad ,Methanol , Double distilled water was purchased from Merck specialties private limited, Mumbai Marketed FormulationsAllrite DC was purchased from the local Market . The instrument Elico LI 120 pH meter, 1.5LH Ultrasonic bath sonicator, Agilent compact LC system.

A. Selection of Sampling Wavelength for Analysis and Preparation of Standard Calibration Curves.

1. Selection of Mobile Phase

The standard solutions containing Levocetirizine and Phenylephrine were injected into the HPLC system and run in different solvent systems. By studying literature survey, different mobile phases in different proportions and different pH were tried in order to find the best conditions for the separation. It was found that methanol and water give satisfactory results as compared to other mobile phases. This mobile phase system was tried with different proportions and using different flow rates. Finally, the optimal composition of the mobile phase was determined to be Methanol: Water (50:50).

2. Preparation of Mobile Phase

The mobile phase was prepared by mixing methanol and water in the ratio of 50:50 and was initially filtered through 0.45µm millipore membrane filter and sonicated for 15 min before use.

3. Preparation of Standard Stock Solution

The separate stock solutions of LEV and PHE were prepared by accurately weighing 10 mg each nto a separate 10 mL volumetric flasks A and B and made up to the volume with mobile phase to get 1000µg/mL respectively. From the above standard stock solutions, 1mL from volumetric flask A and 0.5 mL from volumetric flask B was transferred to a 10 mL volumetric flask and made up to the volume with same mobile phase to get 100µg/mL and 50µg/mL of ABA and LAM respectively (Working stock solution).

4. Selection of Analytical Wavelength

By appropriate dilution of each standard stock solution with the mobile phase, various concentrations of Levocetirizine and Phenylephrine were prepared separately. Each solution was scanned using double beam UV visible spectrophotometer between the range of 200 nm to 400 nm, and their spectra were overload. From the overlain spectra shown in figure 2of Levocetirizine and Phenylephrine, 277 nm was selected as the analytical wavelength for Multicomponent analysis using HPLC method.

5. Optimized Chromatographic Conditions

The mobile phase consisting of Methanol: Water (50:50 v/v) was used inSocratic mode. The mobile phase was initially filtered through 0.45µm millipore membrane filter and sonicated for 15 min before use. The flow rate was maintained at 1 mL/min, and the injection volume was 20µL. UV detection was performed at 277 nm, and the separation was achieved at ambient temperature.

Selection of analytical concentration range and construction of calibration curve for Levocetirizine and Phenylephrine

Appropriate aliquots ranging from 0.5 mL to 2.5 mL were pipetted out from the working stocks olution (100 µg/mL of Levocetirizine and 50µg/mL of Phenylephrine) into a series of 10 mL volumetric flasks.The volume was made upto the mark with the mobile phase to get a set of solution shaving the concentration range, ranging from 5-25 µg/mL of Levocetirizine and 2.5-12.5 µg/mL of Phenylephrine respectively. Triplicatedilutionsof reach of the above mentioned concentrations were prepared separately and from these triplicate solutions,20µLof ieach concentration of the drug were injected into the HPLC system three times separately and their chromatograms were recorded under the same chromatographic conditions as described above.

Peak areas were recorded for all the peaks and a standard calibration icurve of area against concentration was plotted as a concentration of the drug Vs peak area The results were shown in table 24. Both the drugs follow the Beer‘s Lambert‘s law in the concentration range of 5-25 µg/mL of Levocetirizine and 2.5-12.5 µg/mL of Phenylephrine. The linearity of calibrationcurves and adherence of the system to Beer‘s Lambert‘s law was validated by high value of correlation coefficient and less than 2% per cent relative standard deviation (%RSD) for the intercept value which were shown in table 2.

B. Analysis of Tablet Formulation

The tablets (Abamune-L) were initially powdered, and an amount equivalent to 100 mg of Levocetirizine and 50 mg of Phenylephrine was accurately weighed into a 100 mL volumetric flask, mixed with 50 mL of the mobile phase. The solution was made up to the volume with mobile phase and sonicated for 5 minutes. The solution was then filtered through 0.45µm millipore membrane filter. Final stock containing 20µg/mL and 10µg/mL of Levocetirizine and Phenylephrine respectively was prepared by subsequent dilution with the same mobile phase. 20µL of sample solution was injected into the chromatographic system and the peak responses were measured. The solution was injected three times into the column. The amount present in each tablet was calculated by comparing the areas of test with that of the standard. A typical chromatogram of test solution containing 20 µg/ml of Levocetirizine and 10 µg/ml of Phenylephrine was shown in figure 8. The results were shown in table 4.

C. Method Validation [15-20]

The method was validated according to ICH Q2 B guidelines for validation of analytical procedures in order to determine system suitability, linearity, sensitivity, precision, accuracy and robustness for the analytes (ICH Q2B, 1996).

1. System Suitability

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from six replicate injections for Levocetirizine and Phenyle phrineretention times and peak areas.

System suitability was carried out by injecting 100% concentration(sample having 25µg/mL of Levocetirizine and 12.5 µg/mL of phenylephrine) into the HPLC system. This was repeated for six

times under similar condition. thetailing factor i(T) and no. of theoretical plates i(N) obtained were shown in figure 1andthe results were given in table 1.

2. Accuracy

To confirm the accuracy of the proposed method, recovery experiments were performed by standard addition technique. In this method a known quantity of pure drug was added at three different levels i.e. 80 %, 100% and 120% to pre-analyzed sample solutions and calculated the recovery of Levocetirizine and Phenylephrine for each concentration. the results of recovery studies by proposed method were validated by statistical evaluation and were given in table 5.

3. Linearity and Range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. Linearity of the method was determined by means of calibration curve using different concentration of thedrugs. Linearity was evaluated by visual inspection of a calibration curve shown in figure 4and5. The linearity of the method was determined in concentration range of 5-25 µg/mL for Levocetirizine and i2.5-12.5 µg/mL for Phenylephrine. Each solution was injected in triplicate. The slope, intercept was reported as required by ICH which were given in table 02

4. Precision

The precision of an analytical method was studied by performing intraday and inter day precision.

Intraday Precision

Variation of results within thesame day was analyzed. Intraday precision was determined by analyzing a set of six combinedstandardsolutionsof Levocetirizine (25 µg/mL) andPhenylephrine (12.5 µg/mL) in linearity range as 100% concentration at three different time intervals on same day.The results were given in table 07.

Inter day Precision Variation of results between the days was analyzed. Inter day precision was determined by analyzing a set of six combined standard solutions of Levocetirizine (25 µg/mL) and Phenylephrine (12.5 µg/mL) in linearity range as i100% concentration on three consecutive days. The results were given in table 07.

5. Specificity and Selectivity

The specificity of the RP-HPLC method was determined by complete separation of Levocetirizine and Phenylephrine with parameters like retention time (Rt), resolution (Rs) and tailing factor (Tf). Here tailing factor for peaks of Levocetirizine and Phenylephrine was less than 2% and resolution was also more than 2%. The average retention time and standard deviation for Levocetirizine and Phenylephrine were found to be satisfactory for six determinations of sample solution containing 25µg/mL of Levocetirizine and 10µg/mL of Phenylephrine respectively. The peaks obtained for Levocetirizine and Phenylephrine were sharp and have clear baseline separation as none of the excipients interfered with the analytes of interest. The chromatogram to represent specificity was shown in figure 9 and the results were given in table 8.

6. Robustness

The evaluation of robustness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of analysis with respect to deliberate variations in method parameters like different column temperate, different analytical wavelength, different flow rate. the solution containing 25 µg/mL of Levocitrazine sulpahte and 12.5 µg/mL of Phenylephrine was injected into sample injector of HPLC three times under different parameters like deliberate variations in flow rate (±0.1mL/min) and detection wavelength (± 2 nm). Change in flow rate andthe results were given in table 9. for change in detection wavelength andthe results were given in table i10.

7. Ruggedness

The evaluation of ruggedness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of analysis with respect to deliberate variations in method parameters like different instruments, analysts, laboratories, reagents, days etc. the solution containing 25 µg/mL of Levocetirizine and 12.5 µg/mL of Phenylephrine was injected into HPLC three times under different parameters like different analysts. for change in analysts andthe results were given in table 11.

8. LOD andLOQ

The LOD and LOQ values were determined by the formulae LOD = 3.3 σ/S and LOQ = 10 σ/S (Where, σ

is the standard deviation of theresponses and S is mean of the slopes of the calibration curves). theresults were given in table 03.

RESULTS AND DISCUSSION

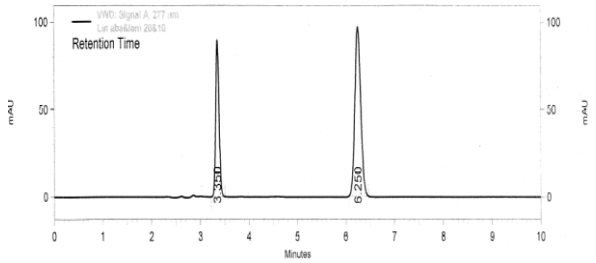

Figure 03: optimized chromatogram for the Levocitrizine, Phenylephrine

Table1: System suitability parameters of Levocitrizine, Phenylephrine

Parameters	Levocetirizine	Phenylephrine
Retention Time (min)	6.25	3.35
Resolution (Rs)	3.0	
Tailing Factor (T)	1.2	1.4
Theoretical Plates (N)	11456	10366

Table 02: Calibration Parameters of Levocetirizine, Phenylephrine

S. No	Levocetirizine			Phenylephrine		
	Conc. (µg/mL)	Rt (min)	Peak Area	Conc. (µg/mL)	Rt (min)	Peak Area
0	0	0	0	0	0	0
1	5	6.2	3644264	2.5	3.3	1941262
2	10	6.2	6570423	5	3.3	3498562
3	15	6.2	10025283	7.5	3.3	5228345
4	20	6.2	13025471	10	3.3	7072569
5	25	6.2	16246592	12.5	3.3	8734279

Figure 04: RP-HPLC Chromatogram of Levocetirizine

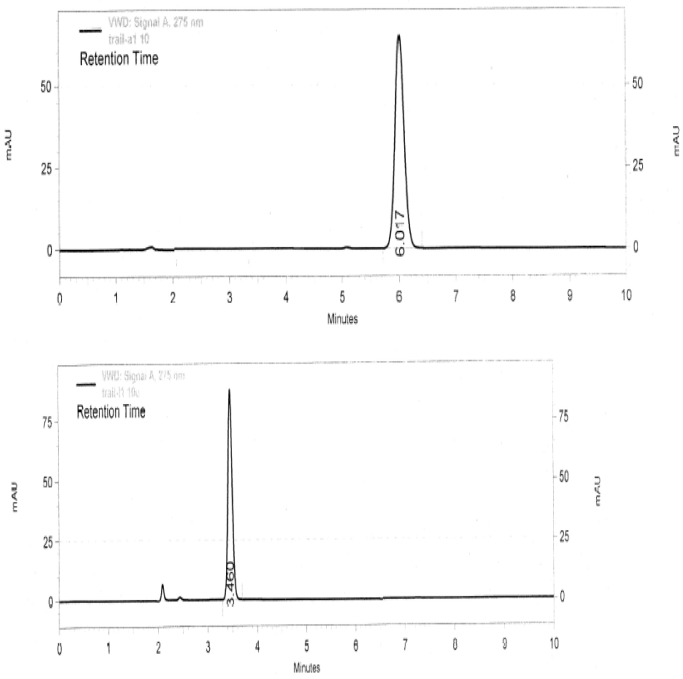

Figure 05: RP-HPLC Chromatogram of Phenylephrine

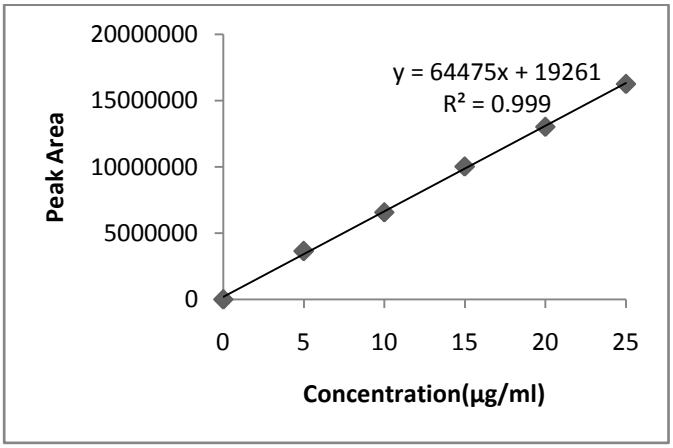

Figure 06: Calibration Curve of Levocetirizine at 277 nm by RP−HPLC Method

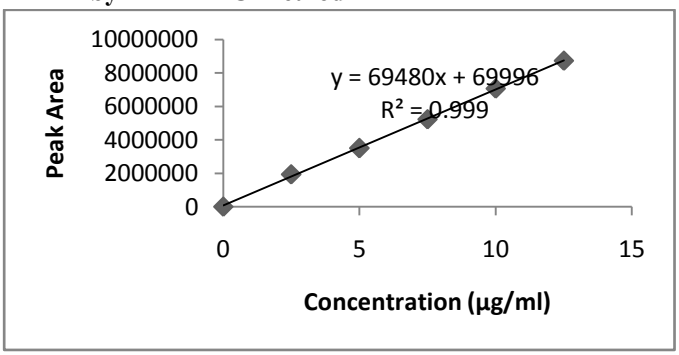

Figure 07: Calibration Curve of Phenylephrine at 277 nm by RP−HPLC Method

Table 03: Statistical Data of Levocetirizine and Phenylephrine at 277 nm by RP–HPLC Method

Parameter	LevocitrizineSulpahte	Phenylephrine
Linearity Range (µg/mL)	5-25	2.5-12.5
Regression Equation	Y=644751x+192617	Y=694801x+69996
Slope (m)	644751	694801
Intercept (c)	192617	69996
Regression Coefficient (r2)	0.9993	0.9997
Limit of Detection (µg/mL)	0.16	0.33
Limit of Quantitation (µg/mL)	0.49	1.01

ASSAY

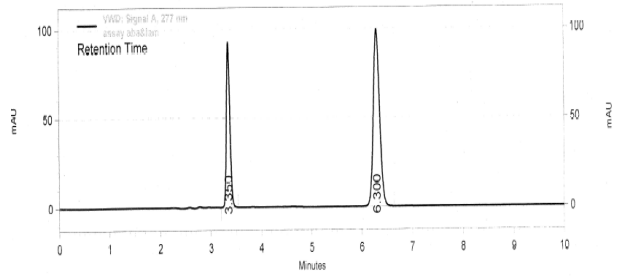

Figure 08: RP-HPLC Chromatogram of Test Formulation

Table 04: Assay of Levocetirizine and Phenylephrine in Tablet Formulation

S. No	Amount Present in (mg/tab)		Amount Obtained in (mg/tab)		Label Claim %w/w	
	LEV	PHE	LEV	PHE	LEV	PHE
1	5	10	4.95	10.1	99	101
2	5	10	5.01	9.98	100.2	99.8
3	5	10	4.99	9.89	99.8	98.9
Mean			4.98	9.97	99.96	99.9
SD			2.9085	1.9194	0.4660	0.5551
%RSD			0.468	0.636	0.466	0.552

ACCURACY

Table 05: Accuracy for Levocetirizine and Phenylephrine

S.No	Recovery Level	Levocetirizine				Phenylephrine			
		Amount Added (µg/mL)		Amount Found (mg)	% Recovery	Amount Added (µg/mL)		Amount Found (mg)	% Recovery (w/w)
		Std.	Test			Std.	Test		
1	80%	8	10	4.95	99	4	5	9.95	99.5
2		8	10	4.98	99.6	4	5	9.98	99.8

3		8	10	5.01	100.2	4	5	10.1	101
4	100%	10	10	5.01	100.2	5	5	10.2	102
5		10	10	5.05	101	5	5	9.98	99.8
6		10	10	4.95	99	5	5	9.97	99.7
7	120%	12	10	5.04	100.8	6	5	9.95	99.5
8		12	10	4.99	99.8	6	5	9.96	99.6
9		12	10	4.92	98.4	6	5	9.97	99.7

Table 06: Statistical Validation Data for % Recovery Determinations

Level of % Recovery	Levocetirizine			Phenylephrine		
	Mean	SD	%RSD	Mean	SD	%RSD
80	99.3	0.814	0.819	98.4	0.642	0.652
100	99.4	0.642	0.646	101.2	0.305	0.301
120	100.2	0.585	0.584	99.5	0.850	0.854

PRECISION

Table 07: Determination of Precision for Levocetirizine and Phenylephrine by RP-HPLC

S. No	Levocetirizine		Phenylephrine	
	Peak Areas			
	Intra day	Inter day	Intra day	Inter day
1	16147878	15036416	8701823	8605248
2	16041854	15294672	8589857	8794482
3	16411866	15629990	8584943	8734279
4	16246592	15311383	8571319	8662145
5	16072779	15388356	8568665	8798391
6	16215954	15333986	8554585	8768665
Mean	16189487	15332467	8595199	8727202
SD	134581.9	190349.7	53714.44	78062.7
% RSD	0.831	1.241	0.624	0.894

SPECIFICITY

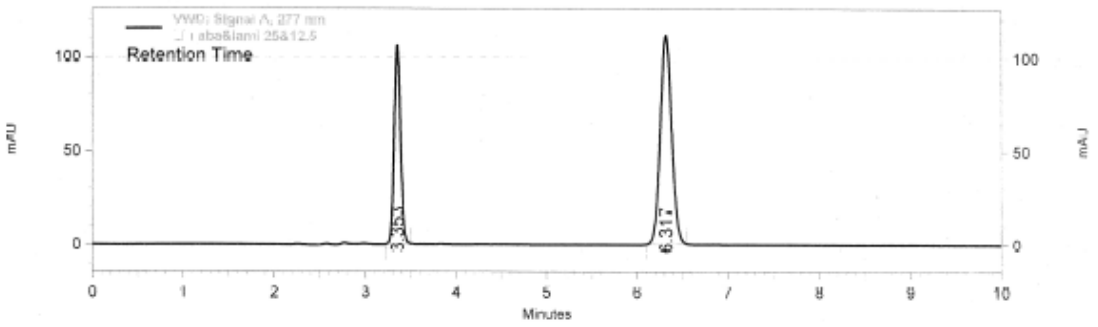

Figure 09: RP-HPLC Chromatogram to Show Specificity of Sample Solution

Table 08: Specificity Parameters of Levocetirizine and Phenylephrine by RP-HPLC

Parameters	Levocetirizine	Phenylephrine
Retention Time (min)	6.31	3.35
Resolution (Rs)	3.0	
Tailing Factor (T)	1.17	1.35
Theoretical Plates (N)	11750	10587

ROBUSTNESS

Table 09: Robustness Data with Change in Flow Rate

S. No	Flow Rate (±0.1mL)	Levocetirizine		Phenylephrine	
		Rt(min)	Peak Area	Rt(min)	Peak Area
1	0.9	3.8	15036416	6.9	8185320
2	0.9	3.7	14965852	7.01	8201356
3	0.9	3.8	15162532	6.8	8126548
Mean		3.7	15054933	6.90	8171075
SD		0.057	99638.97	0.105	39385.99
% RSD		1.532	0.661	1.521	0.482
1	1.1	2.9	14652068	5.8	8835268
2	1.1	3.0	14756825	5.9	8725698
3	1.1	2.9	14865922	5.9	8854256
Mean		2.93	14758272	5.8	8805074
SD		0.057	106934.3	0.057	69394.15
% RSD		1.968	0.724	0.984	0.788

Table 10: Robustness Data with Change in Detection Wavelength

S. No	Detection wavelength (±0.1nm)	Levocetirizine		Phenylephrine	
		Rt (min)	Peak Area	Rt (min)	Peak Area
1	275	6.29	14434097	3.34	8828159
2	275	6.30	14358174	3.35	8795685
3	275	6.34	14478514	3.39	8752352
Mean		6.31	14423595	3.36	8792065
SD		0.026	60853.5	0.026	38032.9
% RSD		0.419	0.421	0.787	0.432
1	279	6.29	15988374	3.34	8898391
2	279	6.32	16072779	3.32	8794482
3	279	6.31	16215954	3.40	8862145
Mean		6.30	16092369	3.35	8851673
SD		0.015	115047.8	0.041	52740.14
% RSD		0.242	0.71	1.24	0.595

RUGGEDNESS

Table 11: Ruggedness Data of Levocetirizine and Phenylephrine

S. No.	Condition	Levocetirizine		Phenylephrine	
		Rt	Peak Area	Rt	Peak Area
1	Analyst-1	6.40	15629990	3.37	8605248
2		6.39	15162532	3.37	8794482
3		6.38	15388356	3.34	8735241
Mean		6.39	15393626	3.36	8711657
SD		0.01	233773.6	0.017	96796.34
%RSD		0.156	1.518	0.515	1.111113
4	Analyst-2	6.43	15253688	3.39	8571319
5		6.41	15162532	3.40	8662145
6		6.40	15036416	3.38	8568665
Mean		6.413333	15150879	3.39	8600710
SD		0.01527	109103.8	0.01	53221.11
%RSD		0.238	0.720	0.294	0.618

DISCUSSION

In HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried to separate title ingredients.The objective of this study was to develop a rapid and sensitive RP-HPLC method for the analysis of Levocetirizine and Phenylephrine in bulk drug and pharmaceutical dosage form by using the most commonly employed C-18 column with UV-detection.

Initially, various mobile phase compositions were tried to elute the drug. Mobile phase ratio and flow rate were selected based on peak parameters (height, capacity, theoretical plates, tailing or symmetry factor), run time andresolution. The system with Methanol: Water (50: 50 v/v) and1 mL / min flow rate was selected.

The optimum wavelength selected was 277 nm from the overlain spectra obtained at which better detector response for the drug was obtained. The retention time for Levocetirizine and Phenylephrine was found to be 6.25min and 3.25 min respectively which were shown in figure 3. The linearity was observed in concentration range of 5-25 µg/ mL and 2.5-12.5 µg/ mL for Levocetirizine andPhenylephrine respectively. Calibration curves of the respective drugs were shown in figure 4 and 5. Summary of validation parameters were given in table 12.

System suitability was assessed by injecting 5replicate injections of 100% test concentration. Number of theoretical plates was more than 2000 for both thedrugs and tailing factor was less than 1.5 for both Levocetirizine and Phenylephrine was reported. A Resolution of greater than 2 was observed. The relative retention times of six replicate injections and system suitability parameters were given in table 01.

The low % RSD values (≤ 2) indicate that the method was precise andaccurate. the mean recoveries were found in the range of 99.3 – 100.2% w/w.

Specificity of the chromatographic method was tested by injecting sample concentration prepared from marketed formulation. The response was compared with that obtained from the standard drug. The chromatogram confirms the presence of Levocetirizine and Phenylephrine at 6.25 min and 3.35 min respectively without any interference. Thus the developed method was specific to Levocetirizine and Phenylephrine and the parameters were given in table 08. The robustness of an analytical method was determined by analysis of aliquots from homogenous lots by differing physical parameters such as change in flow rate to 1.0 ± 0.1 mL and changing detection wavelength 277nm ± 2nm. The

obtained values were given in table 9 and table 10 these values with low % RSD (<2) indicated that the method was quite robust.

Ruggedness of theproposedmethod was determined by analysis of aliquots from homogeneous lot by different analysts, using similar operational andenvironmental conditions; the% RSD reported was found to be less than 2 andthese values were listed in table 11.

The proposed method was validated in accordance with ICH parameters and was applied for analysis of the same in marketed formulations. the content of each component in the formulation was estimated by comparing the peak area of the test sample with that of the peak area of thestandard andthe results were given in table 26 which were found to be 99.83% w/w for Levocetirizine and 100.53% w/w for Phenylephrine respectively. High i%recovery and low % RSD suggested that the method can be applicable for the routine analysis of commercial formulations.

Hence, the developed HPLC method can be adopted for the routine analysis of Levocetirizine and Phenylephrine in pharmaceutical formulations.

SUMMARY

Drug combinations are commonly used clinically and analyst is required to develop suitable methods of their analysis. For routine analytical purposes it is always of interest to establish methods capable of analyzing a large number of samples in a short time period with good accuracy and precision. The commonly used tests of pharmaceutical analysis generally entail compounda testing method development, setting specifications, and method validation.

Analytical testing is one of the more interesting ways for scientists to take part in quality process by providing actual data on the identity, content and purity of the drug products. New methods are now being developed with a great ideal of consideration to worldwide harmonization. As a result, new products can be assured to have comparable quality and can be brought to international markets faster.

A liquid chromatographic technique coupled with spectrophotometric analysis is a versatile tool that can generate extensive analytical data that is highly useful in the routine drug analysis. For routine analytical purposes it is always of interest to establish methods capable of analyzing a large number of samples in a short time period with good accuracy and precision.

In the present work, an attempt was made to provide newer, simple, accurate and low cost HPLC methods for the effective quantitative determination of Levocetirizine and Phenylephrine as active pharmaceutical ingredients as well as in pharmaceutical preparations in their single and combined dosage forms, without the interferences of other constituent in combined formulations. Hence it is planned to develop both HPLC method.

Table 12: Summarized Results of RP-HPLC Method

Parameter	Results	
	Levocetirizine	**Phenylephrine**
Detection Wavelength	277	
Rt (min)	3.35	6.25
Beer's Law Range (µg/mL)	5-25	2.5-12.5
Regression Equation	Y= 644751x+192617	Y= 694801x+69996
Correlation Coefficient i(r2)	0.9993	0.9997
% Recovery (w/w)	99.3-100.2%	98.4-101.2%
LOD (µg/mL)	0.16	0.33
LOQ (µg/mL)	0.49	1.01
Assay (% purty) w/w	99.83%	100.53%
Precision		
Intraday Precision	0.83	0.62
Inter day Precision	1.24	0.89
Robustness		
Flow Rate 0.9 mL/min	1.53	1.52
Flow Rate 1.1 mL/min	1.96	0.98
Detection Wavelength i275 nm	0.42	0.43
Detection	0.71	0.59

Wavelength 279 nm		
Ruggedness		
Analyst 1	1.51	1.11
Analyst 2	0.72	0.61

CONCLUSION

Development of methods to achieve the final goal of ensuring the quantity of drug substances and drug products is not a trivial undertaking. The capabilities ofthemethods developed were complementary to each other. Hence they can be regarded as simple, specific and sensitive methods for the estimation of Levocetirizine and Phenylephrine in single and combined pharmaceutical dosage forms. The developed RP-HPLC method was validated according to ICH guidelines and was found to be applicable for the routine analysis of Levocetirizine and Phenylephrine in their single and combined dosage forms. The developed and validated RP-HPLC method was found to be economical due to the use of higher percentage of water as a solvent in mobile phase. The low solvent consumption (1mL/min), along with short analytical run time of less than 10.0 minutes lead to an environmental friendly chromatographic procedure that allows the analysis of a large number of samples in a short period of time. This method has been found to be better than previously reported methods, due to its wider range of linearity, use of readily available mobile phase, lack of extraction procedures. Hence above method can be used in quality control for routine analysis of finished products of Levocetirizine and Phenylephrine simultaneously without any interference.

REFERENCES

1. L.R. Synder and J.J. Kirkland., “Introduction to Modern LIquid Chromatography”., Wiley International Publication., 2nd Edn., Pg.No: 205-207, 1979.
2. M. Alagar Raja, D. Selva Kumar, A. Rai , Y. Satish Kumar, S. Mishra., "RP-Hplc Method Estimation And Its Validation Of Levocetirizine In Tablet Formulation In Bulk And Pharmaceutical Formulations"., International Journal of Biological & Pharmaceutical Research., Vol.3(1) Pg.No: 1-5, 2012.
3. M.E. Schartz and S. Krull., “Analytical Method Devlopment And Validation”., CRC Press .,1st Edn., Pg.No: i25-46, 2004.
4. in. Pradeep, S. Shanta Kumar, P. Rajesh Kumar., "Analytical Method Development and Validation iof Anti-HIV Drug Levocetirizine"., Journal iof Applied Pharmaceutical Science., Vol. 2,(1), Pg.No: 85-89, 2012.
5. P.D. Sethi., “High performance Liquid Chromatography: Quantitative analysis iofPharamceutical formulation”., CBS Publication.,1st Edn., Pg.no: 5-11,141, 2001.
6. P.V. Rajesh, C.P. Karunasree, G. Dharmamoorthy, K. Padmini, CH. Sudeer.,"Development And Partial Validation iof The Phenylephrine Drug n Bulk And Solid Dosage Form By Uv-Spectroscopy"., nternational Journal iof Pharmaceutical Development & Technology., Vol.2(1), Pg.No: 15-19, 2012.
7. R. Lewis, A. White, G. Bartlett., "Simultaneous Determination iofLevocitrizine And Zidovudine From Rat Tissues Using Hplc With Ultraviolet Detection"., Journal iof Chromatography B, Vol. 850 Pg.No: 45-52, 2007.
8. R. inageswara Rao, R. MastanVali, B. Ramachandra, S. SatyanarayanaRaju., "Separation and characterization iof forced degradation products iof Levocetirizine by LC–MS/MS"., Journal iof Pharmaceutical and Biomedical Analysis, Vol. 54 Pg.No: 279-285, 2011.
9. R. Venkatamahesh, D. Dhachinamoorthi. "Visible Spectrophotometric determination of Levocetirizine in Bulk Drug and Tablet Dosage Form." International Journal of PharmTech Research 3, no. 1 (2011): 356-359.
10. S.K. Patro, S.R. Swain, V. J. Patro , N. S. K. Choudhury. "Development and Validation of High Performance Liquid Chromatographic Method for Determination of Phenylephrine from Pharmaceutical Preparation"., E-Journal of Chemistry., Vol. 7(1) Pg.No: 117-122, 2010.
11. S. Mohidden, G. Vinaykumar, Y. Surendranath, P. Sureshkumar, S. Navaneetha Krishnan., "Validated Rp-Hplc For Simultaneous Estimation Of Levocitrizine And Phenylephrine In Tablet Dosage Form"., International Journal of Pharmacy and Pharmaceutical Sciences., Vol.4(1), Pg.No: 349-355, 2012.
12. S. Surya Rao, I. Khan, G. SrujanaDivya , M. Kumar., "Simultaneous Spectrophotometric

Estimation of Levocetirizine in Tablet Dosage Form"., Archives of Applied Science Research., Vol.2(3), Pg.No: 23-27, 2010.

13. The United State Pharmacopoeia (USP NF), The offcalCompendaof Standards., Unted States Pharmacopeal Convention., Asian Edn.,Pg. No: 2622-2224, 2004.
14. T. Raja, A. Lakshmana Rao., "Development and Validation of RP-HPLC Method for the Estimation ofLevocitrizine, Phenylephrine andZidovudinein Pharmaceutical Dosage Form".,international Journal ofPharmTechiResearch., Vol.3(2), Pg.No: 852-857, 2011.
15. T. Sudha, V.R. Ravikumar P.V. Hemalatha., "Validated HptlcMethod For Simultaneous Determination ofPhenylephrine And Levocetirizine n Tablet Dosage Form".,international Journal ofPharmaceutcialScineces and Research., Vol.1(11) Pg.No: 107-111, 2011.
16. T. Sudha, J. Saminathan, K. Anusha, M. Keerth, Y. Bhargavi, V. Ganesan., "Simultaneous U.V. Spectrophotometric Estimation ofPhenylephrine And Levocetirizine in Bulk And in Tablet Dosage Form"., Journal of Chemical and Pharmaceutical Research., Vol.2(5), Pg.No: 45-51, 2010.
17. V. Singh, L.K. Nath, N.R. Pani., "Development And Validation Of Analytical Method For The Estimation Of Phenylephrine In Rabbit Plasma"., Journal of Pharmaceutical Analysis., Vol.1(4), Pg.No: 251-257, 2011.
18. V.P. Devmurari., "Simultaneous Spectro photometric Determination Of Phenylephrine And Levocitrizine In The Mixture"., International Journal of Pharmaceutical Sciences and Research., Vol.1(7) Pg.No: 82-86, 2010.
19. W. James, M. Robinson Eileen Skelly Frame, M. George Frame., "Undergraduate Instrumental Analysis"., Marcel Dekker,Inc., 6th Edn, Pg.No: 1-3, 2009.
20. Y. Alnouti, C.A. White, M.G. Bartlett., "Determination Of Phenylephrine In Plasma, Amniotic Fluid, And Rat Tissues By Liquid Chromatography"., Journal of Chromatography B, Pg.No: 803 Pg.No: 279-284, 2004.