Available online on www.ijpqa.com
International Journal of Pharmaceutical Quality Assurance 2017; 8(3); 136-147
doi: 10.25258/ijpqa.v8i03.9576

ISSN 0975 9506

Research Article

Development and Validation of Stability Indicating HPLC Method for the Simultaneous Estimation of Levocloperastine Fendizoate and Chlorpheniramine Maleate in Pharmaceutical Dosage Form

Sankalp Patel[1*], Jinal Tandel[1], Samir Shah[2]

[1]*Department of Quality Assurance, Sardar Patel College of Pharmacy, Bakrol, Anand*
[2]*Department of Pharmacology, Sardar Patel College of Pharmacy, Bakrol, Anand*

Received: 28th June, 17; Revised:4th August, 17; Accepted:26th August, 17; Available Online:26th September, 2017

ABSTRACT

The aim of the present study was to develop and validate stability indicating HPLC method for simultaneous estimation of Levocloperastine Fendizoate (LCP) and Chlorpheniramine Maleate (CPM). HPLC method for simultaneous analysis of both drugs was developed and validated according to ICH guideline. Efficient chromatographic separation was achieved on ODS column C_{18} (250 mm × 4.6 mm, 5 µm) using the optimized mobile phase. Stability indicating assay method was carried out by different stress degradation conditions.

In HPLC method, the Retention time for LCP and CPM was 3.173 min and 5.060 min using optimized mobile phase Phosphate buffer (pH-3.5): Methanol (60:40 %v/v) and UV detection at 273 nm. The degradation of LCP, CPM and Formulation was shown to be highest in alkaline condition. Linearity was observed in concentration range of 20-80 µg/ml for LCP and 4-16 µg/ml for CPM. The correlation coefficient of LCP and CPM were respectively 0.9992 and 0.9994. All validation parameters were within the acceptable range. The LOD and LOQ values for HPLC method were found to be 0.146 µg/ml and 0.444 µg/ml for LCP and 0.0113 µg/ml and 0.0344 µg/ml for CPM respectively. The Method validation parameters showed %RSD value less than 2.

Keywords: Levocloperastine Fendizoate, Chlorpheniramine Maleate, HPLC, Stability indicating method, Validation.

INTRODUCTION

Levocloperastine Fendizoate is a cough suppressant. It suppress the cough centers in the central nervous system and directly suppress cough production. Chemically it is 2-[(6-Hydroxy[1,1'-biphenyl]-3-yl)carbonyl]benzoic acid compound with 1-[2-[(S)-(4 chlorophenyl) phenylmethoxy]ethyl]piperidine.

Chlorpheniramine Maleate is a histamine H1 antagonist (or more correctly, an inverse histamine agonist) of the alkyl amine class. In allergic reactions an allergen interacts with and cross-links surface IgE antibodies on mast cells and basophils. Once the mast cell-antibody-antigen complex is formed, a complex series of events occur that eventually leads to cell-degranulation and the release of histamine (and other chemical mediators) from the mast cell or basophil. Once released, histamine can react with local or widespread tissues through histamine receptors. Histamine, acting on H1-receptors, produces pruritis, vasodilatation, hypotension, flushing, headache, tachycardia, and bronchoconstriction. Histamine also increases vascular permeability and potentiates pain. It competes with histamine for the normal H1-receptor sites on effector cells of the gastrointestinal tract, blood vessels and respiratory tract. It provides effective, temporary relief of sneezing, watery and itchy eyes, and runny nose due to hay fever and other upper respiratory allergies.

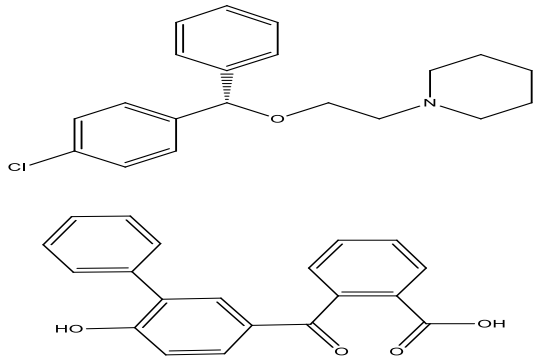

Figure 1: Chemical Structure of Levocloperastine Fendizoate.

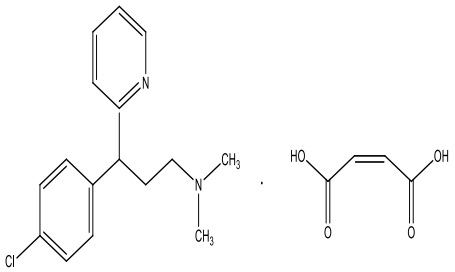

Figure 2: Chemical Structure of Chlorpheniramine Maleate.

**Author for Correspondence: sankalp_020@yahoo.com*

So the mechanism of action of the combination of both drug is synergistic in effect.

Method development should be based on several considerations. It is preferable to have maximum sample information to make development fast and desired for

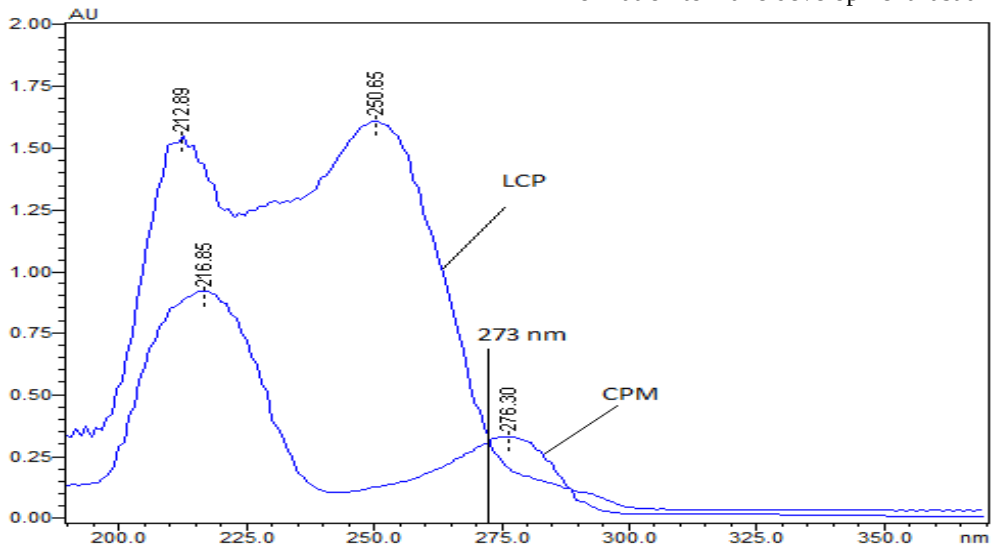

Figure 3: UV Spectra of Levocloperastine Fendizoate and Chlorpheniramine Maleate.

Selection of Chromatographic Condition

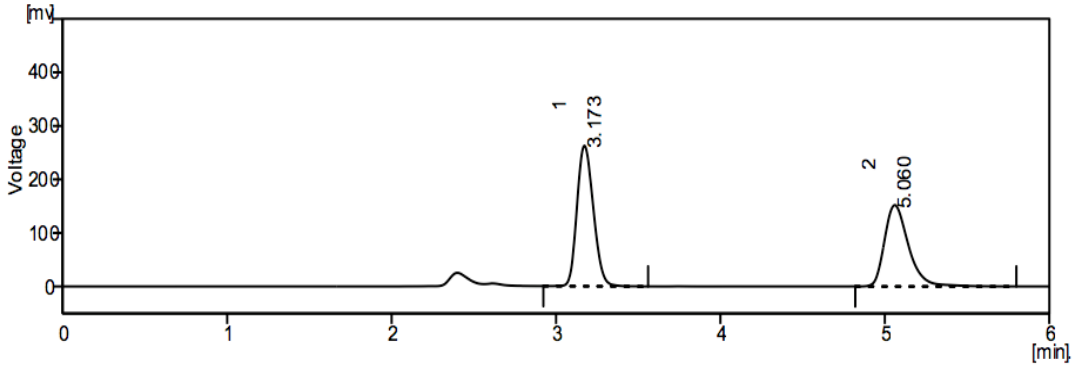

Figure 4: Buffer (pH 3.5) : Methanol (60:40 %v/v).

Force Degradation Study

Acid Degradation

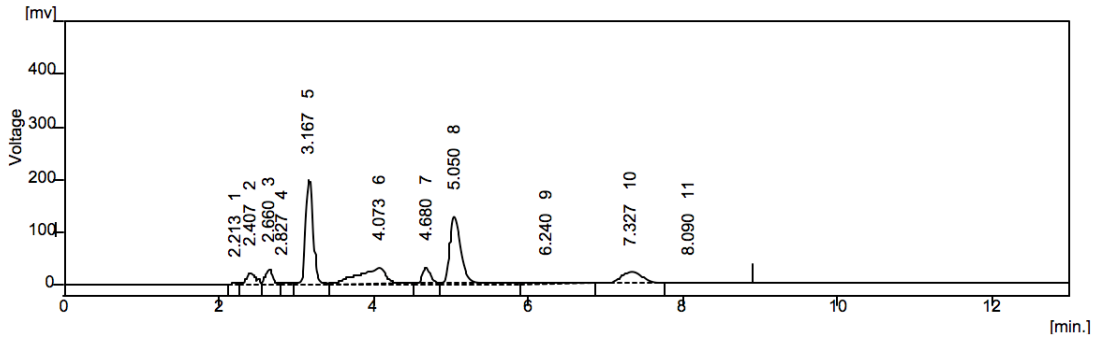

Figure 5: Chromatogram of Acid Degradation (Sample).

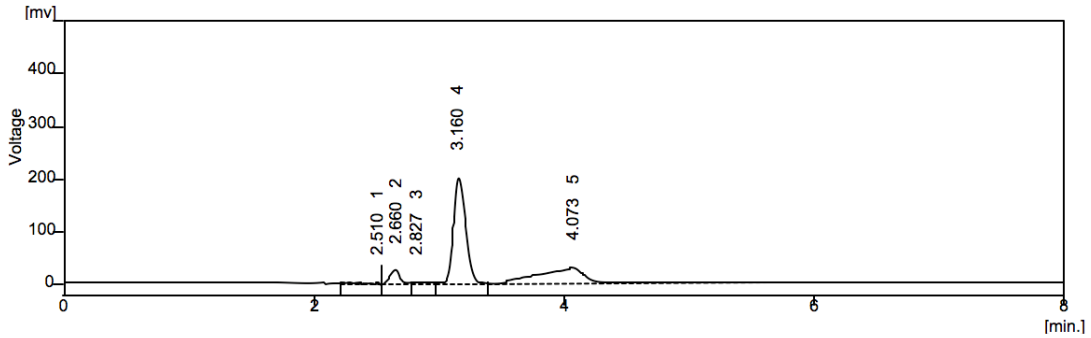

Figure 6: Chromatogram of LCP Acid Degradation.

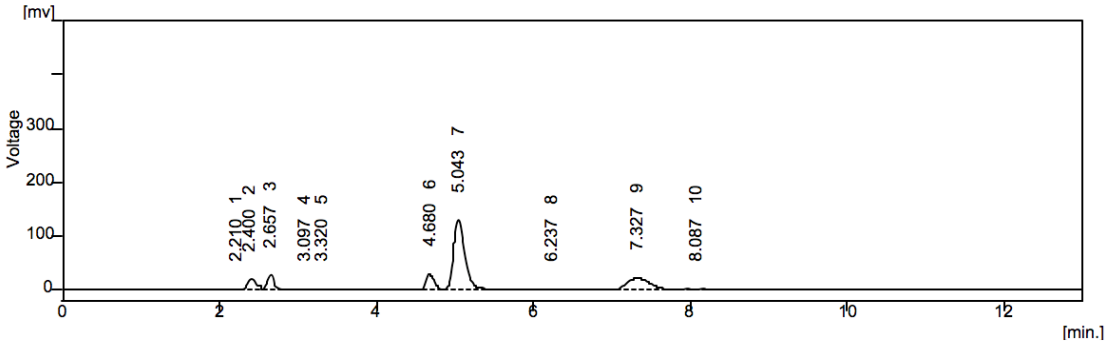

Figure 7: Chromatogram of CPM Acid Degradation.

Base Degradation

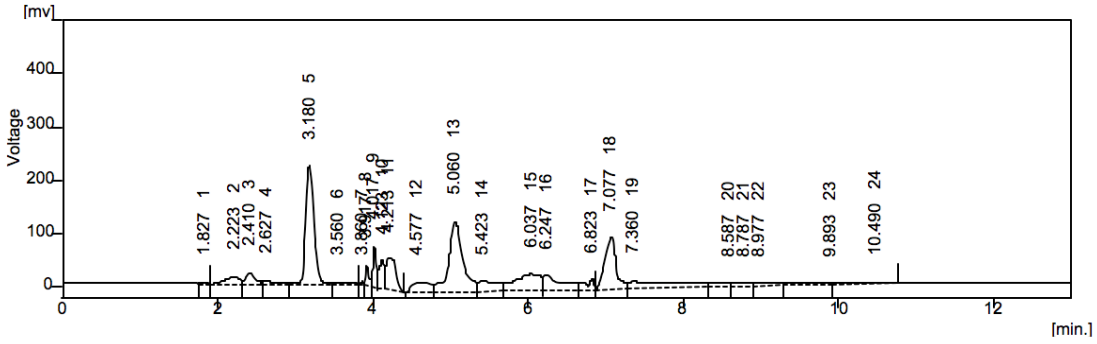

Figure 8: Chromatogram of Base Degradation (Sample).

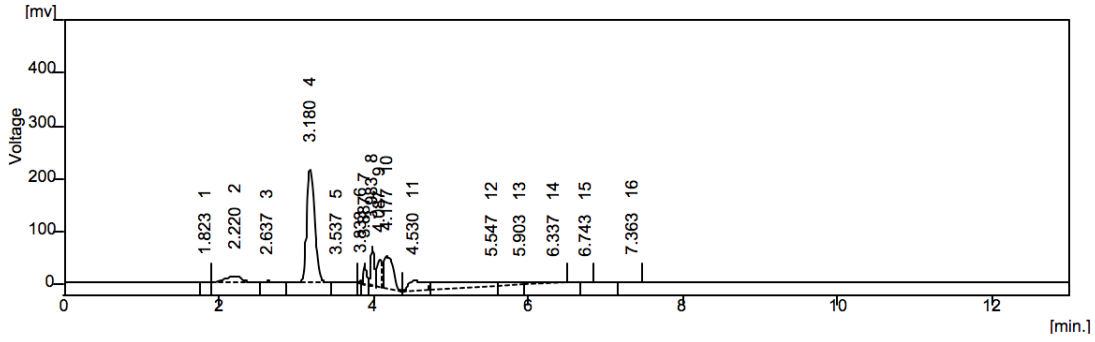

Figure 9: Chromatogram of LCP Base Degradation.

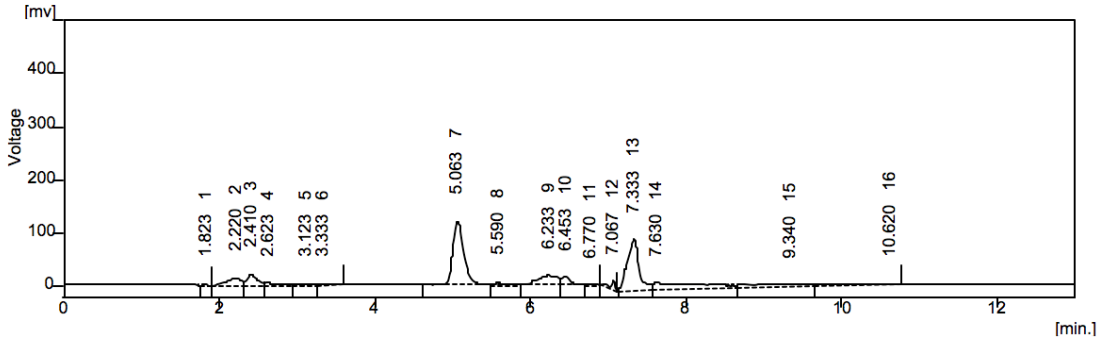

Figure 10: Chromatogram of CPM Base Degradation.

Oxidation Degradation

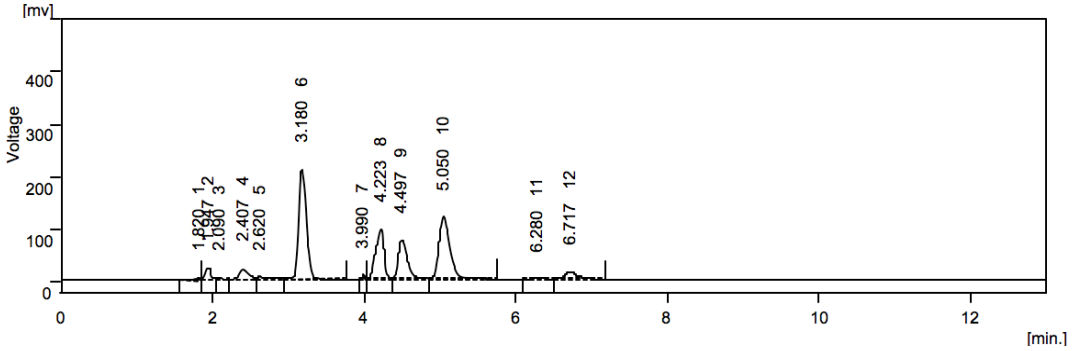

Figure 11: Chromatogram of Oxidation Degradation (Sample).

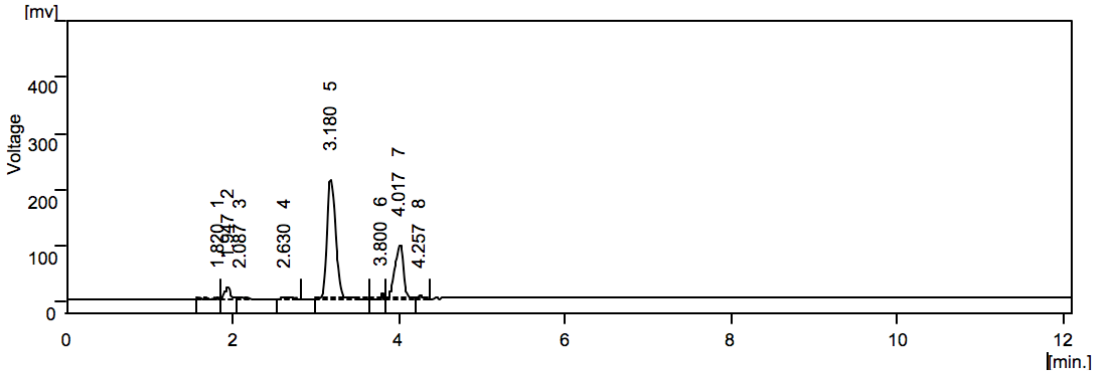

Figure 12: Chromatogram of LCP Oxidation Degradation.

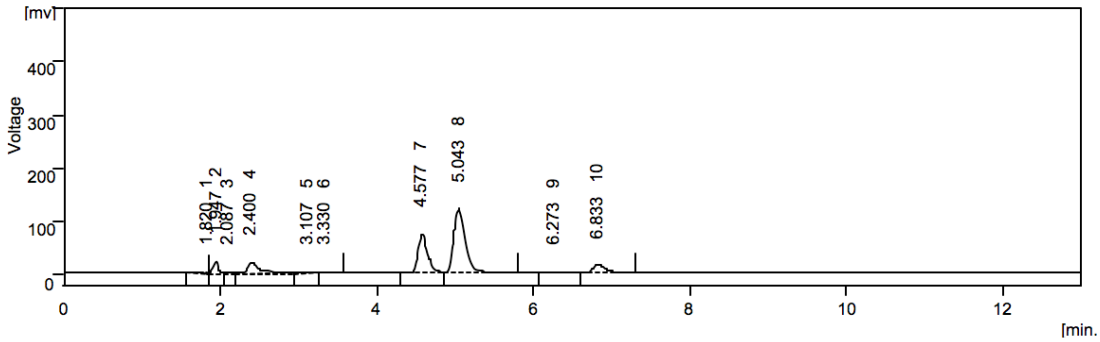

Figure 13: Chromatogram of CPM Oxidation Degradation.

Thermal Degradation

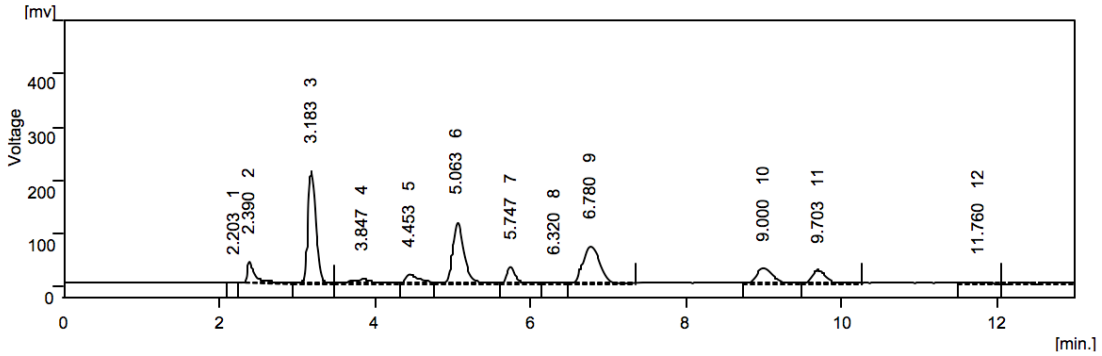

Figure 14: Chromatogram of Thermal Degradation (Sample).

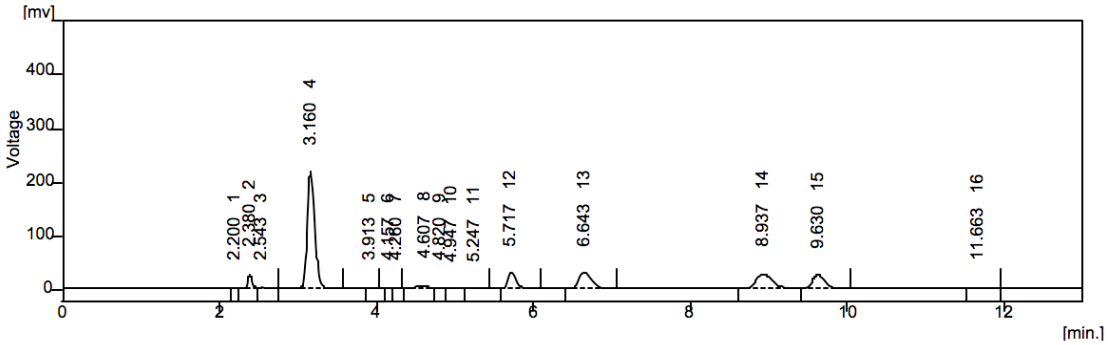

Figure 15: Chromatogram of LCP Thermal Degradation.

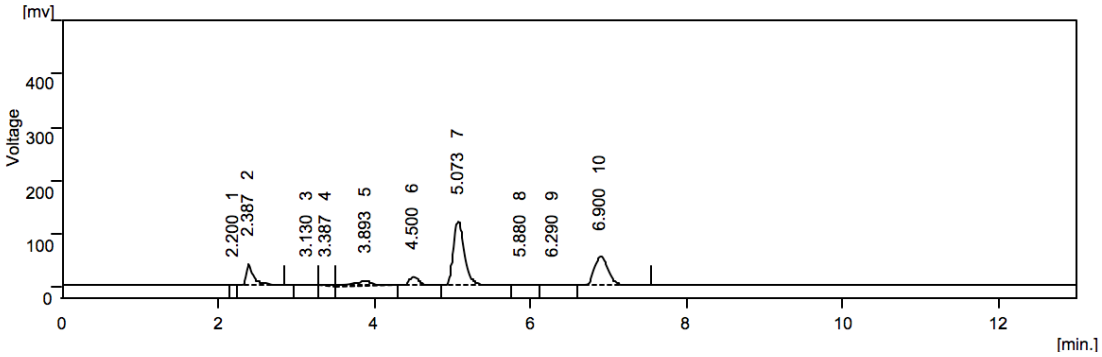

Figure 16: Chromatogram of CPM Thermal Degradation.

Photolytic Degradation

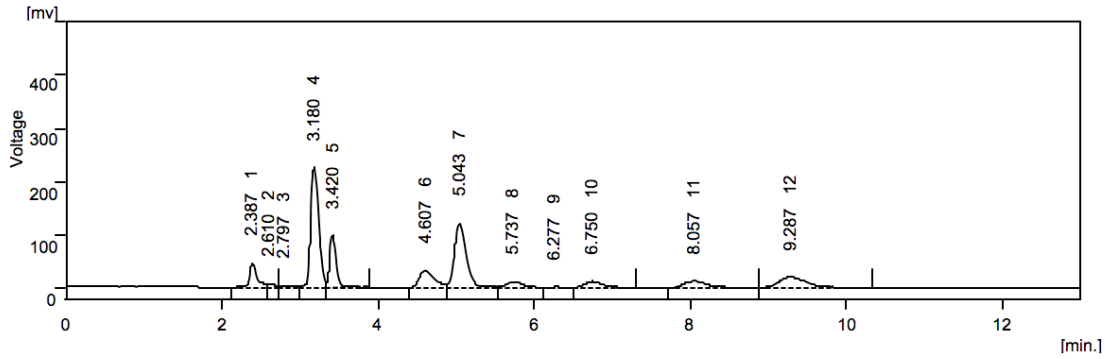

Figure 17: Chromatogram of Photolytic Degradation (Sample).

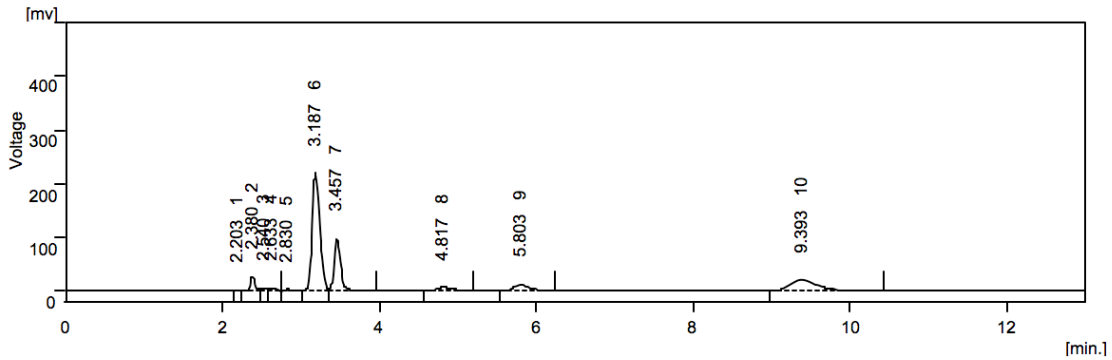

Figure 18: Chromatogram of LCP Photolytic Degradation.

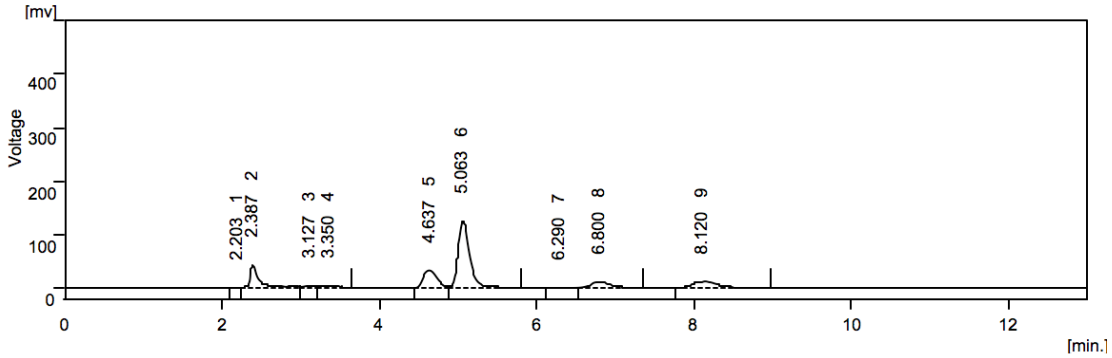

Figure 19: Chromatogram of CPM Photolytic Degradation.

Summary of Force Degradation

Table 1: Summary of LCP and CPM for Degradation.

Sr. No.	Stress Type	% Degradation			
		Standard		Formulation	
		LCP	CPM	LCP	CPM
1	Acidic (0.1N HCl)	17.21	17.61	19.90	19.01
2	Alkaline (0.1N NaOH)	17.46	18.27	19.97	19.81
3	Oxidative (3% H_2O_2)	16.43	17.77	19.95	19.03
4	Thermal (105 °C)	16.71	16.48	19.91	18.79
5	Photolytic (Sun light)	17.26	15.64	19.67	18.09

Table 2: System Suitability Parameters Data of LCP and CPM.

Sr. No.	Parameters	Value Obtained Mean ± SD (n=5)		Standard Value
		LCP	CPM	
1	Retention Time (min)	3.187 ± 0.001	5.043 ± 0.001	-
2	Resolution (R_s)	8.093 ± 0.001		> 2.0
3	Tailing Factor (T_f)	1.360 ± 0.0008	1.543 ± 0.0005	≤ 2.0
4	Theoretical Plate (N)	4380 ± 7.968	5741 ± 2.549	> 2000

Intraday Precision: (n = 3)

Table 3: Intraday Precision of LCP and CPM in HPLC.

LCP			CPM		
Conc. (µg/ml)	Mean Area ± SD	% RSD (σ×100/µ)	Conc. (µg/ml)	Mean Area ± SD	% RSD (σ×100/µ)
20	883.380 ± 5.500	0.622	4	739.599 ± 10.311	1.394
50	1776.795 ± 5.479	0.308	10	1494.101 ± 22.307	1.493
80	2673.604 ± 17.684	0.661	16	2244.838 ± 25.838	1.150

intended analytical method application. Physical and chemical properties are most preferable as primary information. Moreover, separation goal need to be define at beginning so that appropriate method can be developed for the purpose[1,2].

LC method development is widely used for

System Suitability Parameters

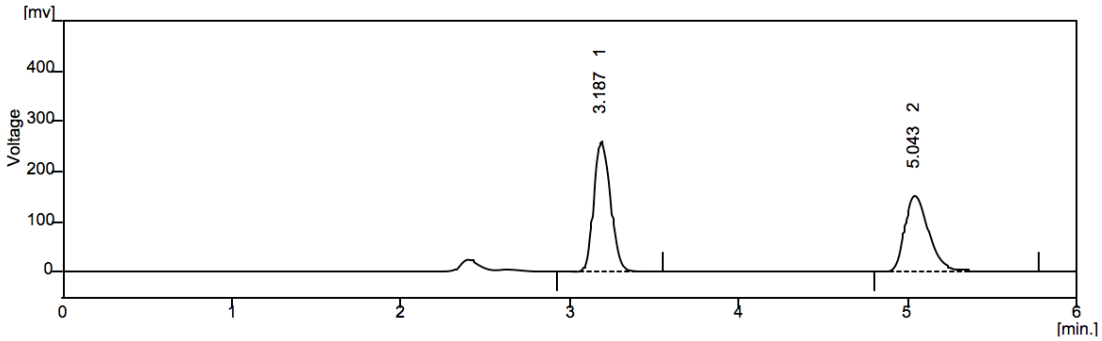

Figure 20: Chromatogram of Standard for LCP and CPM.

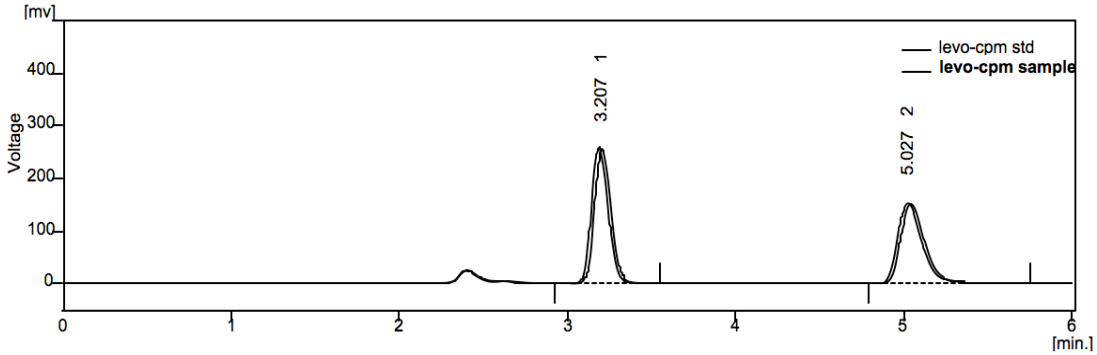

Figure 21: Chromatogram of Standard and sample for LCP and CPM.

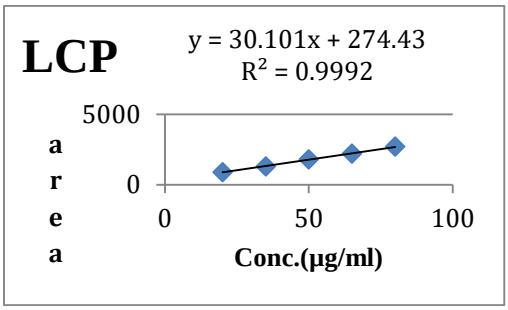

Figure 22: Calibration curve for LCP.

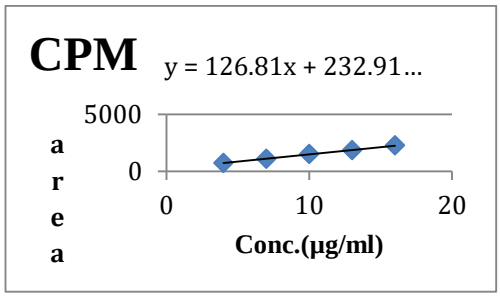

Figure 23: Calibration curve for CPM.

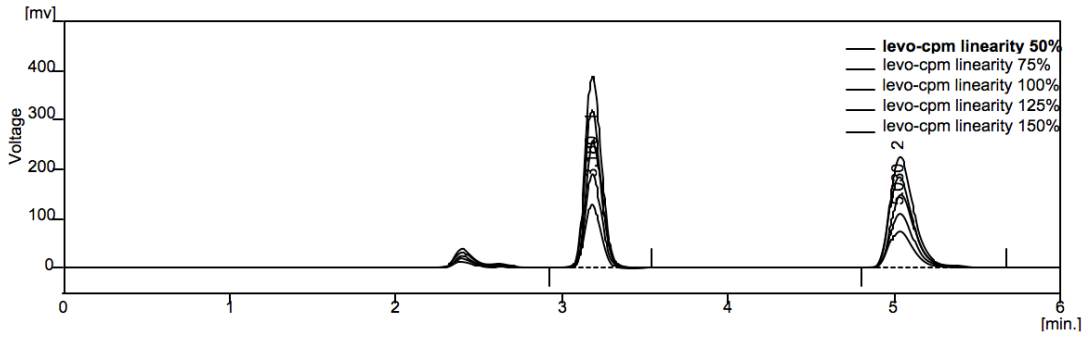

Figure 24: Chromatograph of LCP and CPM.

pharmaceuticals with regulatory requirement of international standards. So, prior to method validation and usage for quality assurance many aspects need to focus as per ICH guidelines[3].

HPLC is separation technique utilizing differences in distribution of compounds to two phases; called stationary phase and mobile phase. The stationary phase designates a thin layer created on the surface of fine particles, and a

Interday Precision: (n=3)
Table 4: Interday Precision of LCP and CPM in HPLC.

LCP			CPM		
Conc. (µg/ml)	Mean Area ± SD	% RSD (σ×100/µ)	Conc. (µg/ml)	Mean Area ± SD	%RSD (σ×100/µ)
20	880.857 ± 5.741	0.651	4	740.066 ± 12.045	1.627
50	1779.177 ± 9.175	0.515	10	1490.312 ± 23.670	1.588
80	2657.088 ± 27.486	1.034	16	2240.361 ± 29.749	1.327

Repeatability: (n=6)
Table 5: Repeatability data of LCP and CPM in HPLC.

LCP			CPM		
Conc. (µg/ml)	Mean Area ± SD	% RSD (σ×100/µ)	Conc. (µg/ml)	Mean Area ± SD	% RSD (σ×100/µ)
50	1789.692 ± 11.954	0.667	10	1503.350 ± 20.889	1.389

Accuracy
Accuracy data for LCP: (n=3)
Table 6: Accuracy data for LCP.

Amount of LCP (µg/ml)	% of STD LCP Added	Amount Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery (Mean ± SD)	% RSD (σ×100/µ)
35	80	28	27.706 28.259 28.045	100.005 ± 0.99	0.991
	100	35	34.684 35.103 34.859	99.664 ± 0.60	0.602
	120	42	42.047 41.706 41.852	99.687 ± 0.40	0.408

Accuracy data for CPM: (n=3)
Table 7: Accuracy data for CPM.

Amount of CPM (µg/ml)	% of STD CPM Added	Amount Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery (Mean ± SD)	% RSD (σ×100/µ)
7	80	5.6	5.586 5.663 5.653	100.616 ± 0.74	0.744
	100	7	6.987 7.018 7.022	100.132 ± 0.27	0.274
	120	8.4	8.389 8.396 8.426	100.048 ± 0.23	0.230

mobile phase designates the liquid flowing over the particles. Under a certain dynamic condition, each component in a sample has different distribution equilibrium depending on solubility in the phases and or molecular size. As a result, the components move at different speed over the stationary phase and thereby separated from each other. The column is a stainless steel (or resin) tube, which is packed with spherical particles. Mobile phase is constantly fed into the column inlet at a constant rate by a liquid pump. A sample is injected from a sample injector, located near the column inlet. The injected sample enters the column with the mobile phase and the components in the sample migrate through it, passing between the stationary and mobile phases[4,5].

Validation of analytical procedures is the process of determining the suitability of a given methodology for providing useful analytical data. Validation is the formal and systematic proof that a method complies with the requirements for testing a product when observing defined procedures. Method validation is primarily concerned with identification of the sources of potential errors and quantification of the potential errors in the method[6,7].
According to FDA guideline, a Stability Indicating Method (SIM) is defined as a validated analytical procedure that accurately and precisely measure active pharmaceutical ingredients (drug substance or drug product) free from process impurities, excipients and degradation products[8].
Stability indicating method must be able to monitor a

Robustness (N=3)

Table 8: Robustness data for LCP and CPM.

Parameters	Variation	LCP		CPM	
		(Mean ± SD)	% RSD (σ×100/μ)	(Mean ± SD)	% RSD (σ×100/μ)
Flow Rate (1 ml/min)	0.8 (ml/min)	1860.766 ± 12.944	0.695	1560.028 ± 17.489	1.211
	1.2 (ml/min)	1751.956 ± 17.714	1.011	1468.816 ± 19.502	1.327
Mobile Phase (60 : 40)	(62 : 38)	1838.303 ± 14.762	0.803	1537.054 ± 28.025	1.823
	(58 : 42)	1745.047 ± 25.055	1.435	1463.827 ± 24.142	1.649
pH	3.3	1840.451 ± 20.382	1.107	1538.462 ± 29.126	1.893
Phosphate Buffer (pH- 3.5)	3.7	1714.256 ± 17.105	0.997	1434.922 ± 24.318	1.694

change in the chemical, physical, and microbiological properties of drug product over time. The ability of the method to monitor a change in the chemical properties of the drug over time, invariably calls for a forced degradation (stress testing) study to be done on the drug substance and drug product[9,10].

Very few analytical method have been reported for chlorpheniramine maleate and levocloperastine fendizoate individually but no combined method has been reported. For chlorpheniramine maleate, two HPLC methods have been published[11], while CPM with other drugs two UV method, RP-HPLC method[12,13], stability indicating RP-HPLC method[14] have been reported. For levocloperastine fendizoate only one UV method has been reported[15].

EXPERIMENTAL

Materials and Reagents

Levocloperastine fendizoate was procured from Lupin LTD., India and Chlorpheniramine maleate was procured from Mahrshree Laboratories PVT. LTD., Vadodara, Gujarat. All HPLC-grade solvent ware obtained from Merck, Rankem.

Chromatographic conditions

A double beam UV- visible spectrophotometer (Systronic, UV-2203) was used. ODS C_{18} column (250mm × 4.6mm, 5μm) with Phosphate buffer (pH-3.5): Methanol (60:40 %v/v) mobile phase. Hamiltone syringe (25 μl) and Systronic- model no. 335 pH meter was used. 1.0 ml min^{-1} flow rate was maintained. An injection volume of 20 μl was injected by means of Rheodyne syringe or injector and UV detection was done at 273 nm. S 1122 HPLC (Analytical Technologies) with Alchrome A 2000 chromatographic software was employed.

Procedure

Preparation of Standard Stock Solutions

Standard Stock Solution of Levocloperastine Fendizoate

50 mg of LCP was accurately weighed and transferred into 100 ml volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution of concentration 500 μg/ml. 1.0 ml of the above solution was transferred to a 10 ml volumetric flask and diluted to the mark with methanol to obtain a working standard solution / standard stoke solution (50 μg/ ml) of LCP.

Standard Stock Solution of Chlorpheniramine Maleate

10 mg of CPM was accurately weighed and transferred to 100 ml volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution (100 μg/ ml). 1.0 ml of the above solution was transferred to a 10 ml volumetric flask and diluted to the mark with methanol to obtain a working standard solution / standard stoke solution (10 μg/ ml) of CPM.

Preparation of Combined Standard Stock Solution of Levocloperastine Fendizoate and Chlorpheniramine Maleate

50 mg of LCP and 10 mg of CPM were accurately weighed and transferred into 100 ml volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution of 500 μg/ ml of LCP and 100 μg/ ml of CPM. 1.0 ml of the above solution was transferred to a 10 ml volumetric flask and diluted to the mark with methanol to obtain a working standard solution of 50 μg/ ml of LCP and 10 μg/ ml of CPM. This solution was used to prepare standard solution for linearity.

Preparation of working standard solution

From the above-prepared stock solution of combined drug (500 μg/ ml LCP, 100 μg/ ml CPM) take 1.0 ml of that solution was taken and made up to 10 ml with methanol. This gave concentration of 50 μg/ ml LCP and 10 μg/ ml CPM.

Preparation of 0.05 M Potassium Dihydrogen Phosphate

6.8 gm of KH_2PO_4 was taken and placed in 1000 ml volumetric flask and dissolved with 1000 mL of water and its pH was adjusted to 3.5 with diluted Orthophosphoric acid.

Forced Degradation Study

Preparation Standard Stock Solution (API)

50 mg of LCP was accurately weighed and transferred to 100 ml volumetric flask, dissolved and diluted up to mark with Mobile phase, to give a stock solution of concentration 500 μg/ml and 10 mg of CPM was transferred to 100 ml volumetric flask, dissolved and diluted up to mark with Mobile phase to give a stock solution of concentration 100μg/ml.

Preparation Stock Solution (For Formulation)

An accurately measured volume of Syrup equivalent to 50 ml of LCP and 10 ml of CPM was transferred into 100 ml volumetric flask. The content was mixed with mobile phase (10 ml), sonicated for 20 min. to dissolve the drug as completely as possible. The solution was then filtering

Assay (n=3)

Table 9: Assay data for Formulation.

Drug	Label Claim (mg)	Batch No.	Avg. Amount found (mg) (Mean ± SD)	Avg. % Assay (Mean ± SD)	% RSD (σ×100/μ)
		M150234	19.638 ± 0.001	98.813 ± 0.558	0.564
		M150235	19.795 ± 0.005	98.975 ± 0.540	0.545
LCP	20	M150237	19.854 ± 0.007	99.258 ± 0.244	0.245
		M150234	4.056 ± 0.002	100.916 ± 1.294	1.282
		M150235	3.978 ± 0.003	99.446 ± 0.025	0.025
CPM	4	M150237	4.076 ± 0.009	101.898 ± 0.022	0.021

through a whatman filter paper no. 41. Volume adjusted up to the mark with mobile phase.

Acid Degradation (0.1 M HCl)

Add 2 ml of 0.1M HCl in standard as well as in reference volumetric flask containing concentration of 50 µg/ml LCP and 10 µg/ml CPM respectively. The volume was made up to 10 ml with mobile phase. After 2 hour neutralize it with 0.1 M NaOH and was studied for acid degradation.

Base Degradation (0.1 M NaOH)

Add 2 ml of 0.1M NaOH in standard as well as in reference volumetric flask containing concentration of 50 µg/ml LCP and 10 µg/ml CPM respectively. The volume was made up to 10 ml with mobile phase. After 2 hour neutralize it with 0.1 M HCl and was studied for base degradation.

Oxidation Degradation (3% H_2O_2)

Add 1mL of 3% H_2O_2 in standard as well as in reference volumetric flask containing concentration of 50 µg/ml LCP and 10 µg/ml CPM respectively. The volume was made up to 10 ml with mobile phase. After 2 hours peroxide degradation was studied.

Thermal Degradation (105 ºC)

Standard stock solution and sample solution of concentration 50 µg/ml of LCP and 10 µg/ml of CPM were taken separately in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase. The solution was stored in oven at 105 ºC for 30 min and Thermal degradation was studied.

Photolytic Degradation (Sun light)

Standard stock solution and sample solution of concentration 50 µg/ml of LCP and 10 µg/ml of CPM were taken separately in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase. The solution was stored under sun light for 5 hours and degradation was studied.

Method Validation

As per ICH guidelines Q2 (R1), the method validation parameters were they are Specificity, Linearity, Precision, % Recovery, Limit Of Detection (LOD), Limit of Quantification (LOQ) and Robustness.

Specificity

Specificity is the ability to measure specifically the analyte of interest without any interference from excipient and mobile phase component. For the determination of specificity 50 µg/ml solution of the standard LCP and 10 µg/ml solution of the standard CPM was injected. Marketed formulation of same concentration was also injected. Both chromatograms were compared and checked for any interference of excipient peak. Chromatogram of blank (only mobile phase) was also recorded to check any interference. Single standard solutions of both drugs were injected for selectivity and peak information.

Linearity (Calibration curve) (n=5)

Mixed working standard solutions (0.4, 0.7, 1, 1.3 and 1.6 ml equivalent to 20, 35, 50, 65 and 80 µg/ml of LCP and 4, 7, 10, 13 and 16 µg/ml of CPM were transferred into a series of 10 ml volumetric flask and diluted to the mark with mobile phase. The solutions of each concentration were injected under the operating chromatographic conditions as described earlier. Chromatograms were recorded. Calibration curves were constructed by plotting peak areas versus concentration and the regression equations were calculated. These operations were done five times and mean responses were calculated. Percent relative standard deviation (%RSD) was calculated.

Precision

Intraday Precision (n=3)

Solution containing 4, 10, 16 µg/ml of CPM and 20, 50, 80 µg/ml of LCP was prepared by transferring 0.4, 1 and 1.6 ml solution from their respective working standard solution containing concentration of 500 µg/ml of LCP and 100 µg/ml CPM.

Interday Precision (n=3)

Take a sample of 0.4, 1.0 and 1.6 ml of working standard solution of LCP (500 µg/ml) and CPM (100 µg/ml) were transferred to a series of 10 ml volumetric flask. The volume was adjusted up to mark with mobile phase to get 20, 50 and 80 µg/ml solution of LCP and 4, 10 and 16 µg/ml of CPM. The area of peaks was measures on the three different days and % RSD were calculated.

Repeatability (n=6)

Solution containing 50 µg/ml of LCP and 10 µg/ml of CPM was prepared. Prepared solution was analyzed 6 times in same day as par the proposed method.

Accuracy (n=3)

It was determined by calculating the recovery of LCP and CPM from formulation by standard addition method. Pre-quantified sample solution of LCP and CPM (35 and 7 µg/ml, respectively) was taken as the 100% of test solution. To a fixed amount of test 80%, 100% and 120% amount of standard was added and the amount of standard to be found was calculated using regression equation. Known amount of standard solutions of LCP (28, 35 and 42 µg/ml) and CPM (5.6, 7 and 8.4 µg/ml) were prepared.

Summary of Validation Parameters

Table 10: Summary of LCP and CPM for Validation.

Sr. No.	Parameters		Results	
			LCP	CPM
1	Linearity (µg/ml)		20-80	4-16
2	Slope		30.101	126.810
3	Correlation Coefficient		0.9992	0.9994
4	Intra-day Precision (% RSD)		0.308-0.661	1.150-1.493
5	Inter-day Precision (% RSD)		0.515-1.034	1.327-1.627
6	Repeatability (% RSD)		0.667	1.389
7	Accuracy (% Recovery)		99.664-100.005	100.048-100.616
8	LOD (µg/ml)		0.146	0.0113
9	LOQ (µg/ml)		0.444	0.0344
10	Robustness	Flow Rate	0.695-1.011	1.211-1.327
	(% RSD)	Mobile Phase Ratio	0.803-1.435	1.823-1.649
		pH	1.107-0.997	1.893-1.694
11	% Assay		98.813-99.258	99.446-101.916

Each solution was injected in triplicate and the percentage recovery was calculated by measuring the responses and putting these values into the regression equations of the respective calibration curves.

System Suitability Test

Solution containing 50 µg/ml of LCP and 10 µg/ml of CPM were prepared. Data obtained from the peak i.e. peak area, retention times, tailing (symmetry) factor, column efficiency (theoretical plates) etc. were recorded. All system suitability parameters were computed using these recorded data.

LOD and LOQ

As per ICH guideline, limit of detection and quantitation of the developed method were calculated from the standard deviation of the response and slope of the calibration curve of each drug using the formula,

$LOD = 3.3 \times \sigma$ / mean of slop[6]

$LOQ = 10 \times \sigma$ / mean of slop[6]

σ = Standard deviation of Response

Robustness

In this parameter, small but deliberate changes were made to check deviation in result. This is done to check how results remain unaffected by small changes that are made. Method robustness was evaluated by changing the flow rate, wavelength and mobile phase composition to evaluates the impact on the performance of the method and the results will be expressed in terms of %RSD.

Analysis of Marketed Formulation

The method was used for simultaneous estimation of LCP and CPM in syrup dosage forms. From syrup withdraw equivalent ml of 50 mg of LCP and 10 mg of CPM was transferred in to 10 ml volumetric flask and 5 ml of mobile phase was added in to it. Solution was solicited for 30 minutes with intermittent shaking, cooled to attain room temperature and added up to 10 ml of mobile phase and mixed well. It was filtered through 0.45-µ-syringe filter. Further 1 ml of the above filtrate was diluted to 10 ml with mobile phase to get 500 µg/ml concentration of LCP and 100 µg/ml concentration of CPM in mixture sample respectively.

RESULTS AND DISCUSSION

Selection of wavelength

Standard stock solutions of Levocloperastine Fendizoate 50 µg/ml and Chlorpheniramine Maleate 10 µg/ml were scanned in UV region of 200-400 nm and the spectrum was recorded. Detection wavelength for Levocloperastine Fendizoate and Chlorpheniramine Maleate was found to be 273 nm.

To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase, pH and solvent ratio were studied. A numbers of different trials were taken and the resulting chromatograms were recorded. These ware the final chromatograms of the phosphate buffer (pH-3.5) : Methanol (60:40 %w/v) mobile phase selected. From these the retation time of LCP is 3.173 min and CPM is 5.060 min.

Force degradation study of LCP, CPM and marketed formulation was done under Hydrolytic, Oxidative, Thermal and Photolytic conditions. In this, both drugs and marketed formulation gives maximum degradation in alkaline hydrolysis, which is < 20 %.

Validation of RP-HPLC Method

From the retention time value of both drugs it was identified that the drug which gave peak at 3.187 is of LCP and the other gave peak at 5.043 is of CPM. The resolution of the peak was 8.093 and it was > 2 and obtained value of tailing factor and theoretical plate ware under standard value.

Specificity

Overlapping the graph of both standard as well as sample drug checked specificity.

Linearity: (n=5)

Linearity study was carried out for both drugs at different concentration levels.

Linearity range of Levocloperastine Fendizoate and Chlorpheniramine Maleate were found to be 20 - 80 µg/ml and 4 - 16 µg/ml.

The R^2 value of LCP and CPM was 0.999.

Precision

Intermediate Precision

I. Intraday

II. Interday

III. Repeatability

CONCLUSION

A Development and validation of Stability Indicating HPLC Method have been developed and validated as per ICH guideline for the simultaneous estimation of LCP and CPM in pharmaceutical dosage form. The Linearity range of LCP and CPM are 20-80 µg/ml and 4-16 µg/ml respectively and correlation coefficient value was found to be 0.999. Intraday, Interday, repeatability and robustness was done and the % RSD value was found < 2. LOD and LOQ of LCP are 0.146 and 0.444 µg/ml and that of CPM are 0.0113 and 0.0344 µg/ml respectively. % Recovery of LCP and CPM lies between 98-102%. % Assay of LCP falls between 98.813-99.258 and of CPM between 99.446-101.916.

REFERENCES

1. Tripathi KD. Essential of Medical Pharmacology. 6th Edition, Jaypee Brothers Medical Publisher, New Delhi, 2010, 215.
2. Rang H, Dale M, Ritter J, Flower R. Pharmacology. 6th Edition, Churchill Livingstone, Elsevier, Philadelphia, 2003, 336.
3. Skoog DA, West DM, Holley FJ. Fundamentals of Analytical Chemistry. 8th Edition, Saunders College Publishing, New Delhi, 2004, 836-864.
4. Snyder L, Kirkland J, Glajch L. Practical HPLC Method Development. 2nd Edition, A Wiley-Inter science publication, NY, USA, 1997, 3-35.
5. Meyer VR. High Performation Liquid Chromatography Practical. 2nd Edition, John Wiley and Son's Publishers, London, 1993, 46-96.
6. International Conference on Harmonization Guideline on Validation of Analytical Procedures (2005) Text and Methodology: Q2 (R1).
7. P. Araujo. Key aspects of analytical method validation and linearity evaluation. Journal of Chromatography B 2008; 877: 2224-2234.
8. Carstensen JT, Rodes CT. Drug stability principle and practices. 3rd Edition, Marlel Delkker, 2008, 245.
9. International Conference on Harmonization Guideline on Stability Testing of New Drug Substances and Products (2003) Q1 A (R2).
10. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drug. Journal of Pharmaceutical Analysis 2014; 4(3): 159-165.
11. Dzambic LA, Vehabovic M, Cekic E, Dzambic M. Development and Validation of a HPLC method for Chlorphenamine Maleate Related Substance in Multicomponents Syrups and tablets. International Journal of Pharmacy Teaching and Practices 2014; 5(3): 997-1001.
12. Bagada H, Panchal H, Patel P, Jani A. Analytical Method Development and Validation for Simultaneous Estimation of Chlorpheniramine Maleate, Ambroxol and Phenylephrine HCl in Bulk and Liquid Dosage Form By RP-HPLC Method. World Journal of Pharmacy and Pharmaceutical Sciences 2014; 3(5): 1458-1467.
13. Aceampong A, Gyasi WO, Darko G, Apau J, Addai-Arhin S. Validated RP-HPLC method for simultaneous determination and quantification of chlorpheniramine maleate, paracetamol and caffeine in tablet formulation. SpringerPlus 2016; 5: 625.
14. Kommana R, Basappa P. Validated Stability Indicating RP-HPLC Method for Simultaneous Estimation of Codeine Phosphate and Chlorpheniramine Maleate from Their Combined Liquid Dosage Form. Chromatography Research International 2013; 7.
15. Jain NA, Umekar MJ, Lohiya RT. Spectrophotometric Method Development for Determination of Levocloperastine Fendizoate in Bulk and Pharmaceutical Dosages Form. Asian Journal of Recearch in Chemistry 2011; 4(8): 1231-1234.