

Research Article



Development and Validation of RP-HPLC Method for Estimation of Alcaftadine in Bulk Drug and Dosage Form

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ABSTRACT

A new, sensitive, suitable, economic, accurate, and robust reversed-phase high-performance liquid chromatography (RP-HPLC) method for the determination of Alcaftadine in bulk drug and ophthalmic formulation was developed and validated in this research. The separation was performed using a HPLC method with a UV detector and Openlab EZchrome workstation programme, Kromasil C18, 250 mm X 4.6mm ID, 5 µm Acetonitrile: 0.1 % OPA (90:10%V/V) with a flow rate of 1.0 mL/min and detected at 282 nm. The developed RP-HPLC method yielded a suitable retention time for Alcaftadine of 3.05 min, which was optimized using trial and error basis. The linearity of the determined method was found a correlation coefficient (r^2) of 0.9999 over the concentration range of 0.5-7.5µg/mL. The percentage RSD for the method's precision was found to be less than 2.0 percent. The percentage recovery was discovered within the limit 0.095 ug/mL and 0.288 ug/mL were found to be the LOD and LOQ, respectively. The developed and validated RP-HPLC system takes less time and can be used in the industry for routine quality control/analysis of bulk drug and marketed Alcaftadine products. In present studies, the retention time was less than previous reported.

Keywords: RP-HPLC, Alcaftadine, Allergic Condition, Method Development, Validation.

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INTRODUCTION

Ocular allergy is a commonly come across pathology in clinical practice, with raised in number of patients noticed in the last decade. Number of sources have been considered for this increase such as genetics, air pollution, pets, etc.¹ Avoidance of allergens and lubrications plays an important role in the management of allergic conjunctivitis. Addition of anti-histaminic such as levocabastine decreases inflammation, whereas mast cell stabilizers help mast cell degranulation on exposure to allergens. Topical corticosteroids are the dominant agents to control inflammatory symptoms, but their use is not devoid of side-effects²⁻³. Recently, introduced topical agents have both anti-histaminic and mast cell stabilization action. Their use can control acute symptoms and prevent relapses as well. These agents (such as olopatadine, Bepotastine, and Alcaftadine) are USFDA approved for use in allergic conjunctivitis in July 2010. It is commercially marketed under the name LASTACAF. ⁴

Chemistry

Chemically Alcaftadine is 6, 11-dihydro-11-(1-methyl-4-piperidinylidene)-5H-imidazo [2,1-b][3] benzazepine-3-carboxaldehyde (Fig. 1). Alcaftadine is a broad-spectrum

antihistamine displaying a high affinity for histamine H1 and H2 receptors and a lower affinity for H4 receptors. It also exhibits modulatory action on immune cell recruitment and mast cell stabilizing effects. It acts by inhibiting release of histamine from mast cells. The drug was approved by USFDA.⁵⁻⁷ The metabolism of Alcaftadine is mediated by non-CYP450 cytosolic enzymes to the active carboxylic acid metabolite. The protein binding of Alcaftadine and the active metabolite are 39.2% and 62.7% respectively.⁸

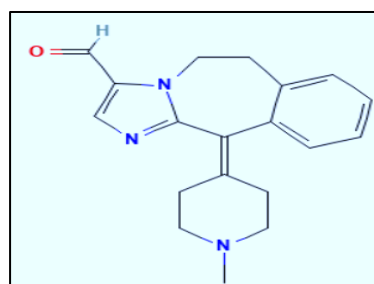


Figure 1: Chemical Structure of Alcaftadine

MATERIALS AND METHODS

Materials

Sample Alcaftadine received as gift sample. Qualigens (Thermo fisher scientific) provided HPLC grade chemicals for the work. The marketed formulation Alcatop (0.25% w/v Eye drop) was procured from drug store.

Instrumentation and software

An Agilent 1260 Infinity II HPLC system with DEAX02386 pump and autosampler with UV-visible detector served as the chromatographic system (DEACX16446). For data collection and processing, the chromatograms were



registered using Openlab EZ Chrome Workstation on a Windows-based computer system. Alcaftadine concentrations were determined using a Kromasil C₁₈ column (250 mm X 4.6 mm i.d. 5µm) column.

Methods

Selection of analytical wavelength

Selection of solvent: Methanol was selected as the solvent for dissolving Alcaftadine. Methanol as a blank and Alcaftadine standard solution (20 PPM) was scanned from 400 nm to 200 nm. Absorption maxima was determined for drug. Alcaftadine showed maximum absorbance at 282 nm shown results in figure 2.

Method Development by RP – HPLC

Preparation of standard stock solution

In order to prepare stock solution, weighed accurately 10 mg Alcaftadine and transferred into 20 ml volumetric flask, added 15 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (500 PPM). Further diluted 2 ml of stock solution to 10 mL with mobile phase (100 PPM).

Selection of analytical wavelength

Analytical wavelength for the examination was selected from the wavelength of maximum absorption from the spectrophotometric analysis and it was 282 nm shown in figure 2.

Method Validation by RP – HPLC

a) Development and optimization of HPLC method: After all experimental trials and with reference to the acceptance criteria for various system suitability parameters, the conditions were optimized for the estimation of Alcaftadine bulk drug and its dosage form and result shown in figure 3 and table 1.

b) Preparation of System suitability test (Alcaftadine standard solution): Weighed about 10.13 mg of Alcaftadine and transferred in 50 mL volumetric flask, added 30 mL of methanol, sonicated to dissolve it, made volume up to the mark with methanol. Pipette out 0.5 ml from standard stock solution and transferred into 20 ml volumetric flask and made volume up to the mark with mobile phase chromatograms were recorded and result shown in figure 3.

A. Analysis of marketed test sample: (Assay)

Marketed test sample Having Name Alcatop 0.25% w/v Eye drop was selected for analysis and for doing validation.

Weight per ml of test sample (Alcatop 0.25% w/v Eye drop):

Calculated weight per mL of test sample by following formula:

$$\text{Weight per mL} = \frac{W_3 - W_1}{W_2 - W_1} \times \text{Density of Water at } 25^\circ\text{C}$$

Sample preparation of Marketed test sample

Weigh accurately 1010 mg from Alcatop eye drop 0.25% w/v equivalent to 2.5 mg of Alcaftadine and transferred to 20 ml of volumetric flask and makeup volume with methanol. Filtered the solution through suitable 0.45 µm syringe filter. Further diluted 1ml of filtered stock solution to 25 ml with mobile phase. Sample prepared in duplicate and result shown in table. 2.

Formula for % Assay calculation

$$\% \text{ Assay} = \frac{\text{Alcaftadine Std area}}{\text{Alcaftadine Std area}} \times \frac{\text{Alcaftadine STD wt (mg)}}{50} \times \frac{0.5}{20} \times \frac{20}{\text{Sample wt (mg)}} \times \frac{25}{1} \times \frac{\text{Weight per ml (mg)}}{\text{Label claim of Alcaftadine (mg per mL)}} \times 100$$

B. Method Validation Parameter

Specificity

Following solution shall be prepared and injected to prove the specificity nature of the method (Checked peak purity for standard and test sample solution). Blank (mobile phase as a diluent), Alcaftadine standard solution, test sample solution and placebo were injected. Analysing marketed test sample contains excipients (additives) which are totally unknown. So, Placebo prepared at lab level by using formula as benzalkonium chloride (0.005%) used as preservative, sodium chloride (0.05%) as tonicity agent, sodium hydroxide (QS to adjust pH 6.3 to 7) as pH modifier and water (QS 100 mL) as diluent.

Placebo Sample solution preparation

Weighed 1007.5 mg of placebo material (Which is equivalent to 2.5 mg of Alcaftadine) and transferred to clean and dried 20 mL of volumetric flask. Added 15 mL of methanol, sonicated for 10 minutes with intermittent shaking. After 10 minutes allow to cool the solution to room temperature and made volume up to the mark with methanol. Filtered the solution through suitable 0.45 µm Nylon syringe filter discarding 3-5 mL of initial filtrate. Further dilute 1 ml of filtered stock solution to 25 ml with mobile phase, injected the resultant solution and chromatograms were recorded.

Linearity and Range

Preparation of linearity solution

The linear response of standard solution was determined over the range of 0.50 to 7.5 µg/mL. The linear response was plotted analyte concentration with respect to peak area. 5 levels of Linearity were performed from 10% to 150% of working concentration.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

As per guidelines ICH Q2R1 LOD and LOQ was determined by using the approach Based on the Calibration Curve in which residual standard deviation of a regression line was calculated and determined the LOD and LOQ by using following formula:

$$\text{LOD} = 3.3 \sigma / S$$



$$LOQ = 10 \sigma / S$$

Where, σ = residual standard deviation of a regression line
 S = Slope of regression line

Accuracy (% Recovery)

Accuracy studies was conducted in the range from 50 %, 100% and 150 % of working concentration. Solution of each accuracy level was prepared in triplicate. Calculated % Recovery for each sample, Mean % recovery for each level and overall recovery and also calculated % RSD for each level and % RSD for overall recovery. Weigh accurately 50 mg of stock solution by dissolving in 20 ml of volumetric flask. Further dilution shown in table.3.

Precision

Precision is of two types, Repeatability and Intermediate precision. It is performed on eye drop test sample.

Repeatability: Weigh accurately 1010 mg of sample solution and transferred into 20 ml of volumetric flask and add methanol up to marked and filtered the solution. Further diluted 1ml of filter stock solution to 25 ml of mobile phase and injected. Six samples were prepared.

Intermediate precision: It is performed by doing analysis on another day to check reproducibility of results. Samples prepared in same manner as that of repeatability parameter.

Robustness

The robustness of an analytical procedure gives an indication of the reliability of the developed method during normal usage. The reproducible results were obtained which proves that method is robust. Standard solutions were injected under different chromatographic conditions as changes in flow rate by $\pm 10\%$. (± 0.1 ml/min), change in column oven temperature. ($\pm 2^\circ\text{C}$) and change in wavelength (± 3 nm).

RESULTS AND DISCUSSION

Selection of analytical wavelength

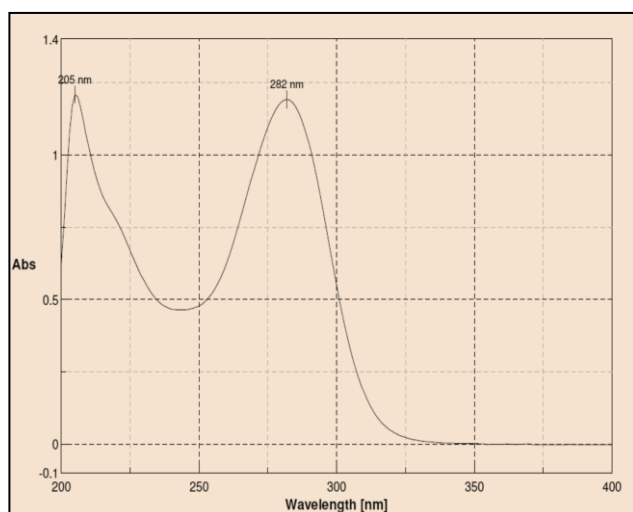


Figure 2: UV spectrum of Alcaftadine solution 20 PPM

Development and optimization of HPLC method

Table 1: Optimized Chromatographic Conditions

Parameter	Description
Mode	Isocratic
Column Name	Kromasil C18, 250 mm X 4.6mm ID, 5 μm
Detector	UV Detector
Injection Volume	20 μl
Wavelength	282 nm
Column Oven temp	40 $^\circ\text{C}$
Mobile Phase	Acetonitrile: 0.1 % OPA (90:10%V/V)
Flow Rate	1.0 ml/min
Run time	6 Minutes
Retention time	3.05 Minutes

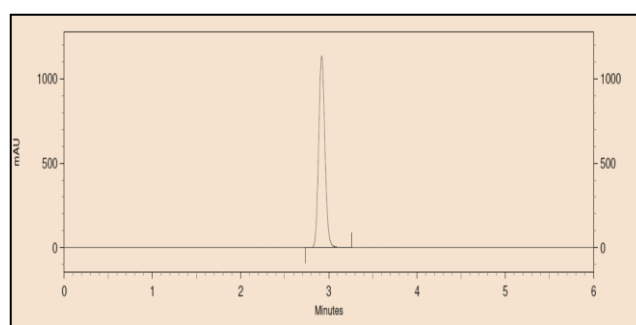


Figure 3: Typical chromatogram of Optimized Trial

System Suitability Test

It was observed from the data % relative standard deviation was not more than 2.0%, theoretical plates more than 2000 and tailing factor not more than 2.0; the method complies with system suitability parameters. Hence, it can be concluded that the chromatographic method is adequate for intended analysis.

Analysis of marketed test samples (Assay)

Alcatop 0.25% w/v Eye Drop:

Weight per mL Calculation:

Weight of empty picnometer (W1): 27.47 gm

Weight of picnometer with water (W2): 37.58 gm

Weight of picnometer with test sample (W3): 37.71 gm

Density of water at 25 $^\circ\text{C}$: 0.99602

$$\text{Weight per mL} = \frac{W3 - W1}{W2 - W1} \times 0.99602$$

$$\text{Weight per mL} = \frac{37.71 - 27.47}{37.58 - 27.47} \times 0.99602$$

$$\text{Weight per mL} = 1.010 \text{ gm / mL}$$

Table 2: Assay results of Alcatop 0.25% w/v Eye Drop

Sample	Area	% Assay	Mean Assay
Sample 1	4898702	100.35	99.46
Sample 2	4820381	98.56	

Validation of RP-HPLC method

The optimized method for estimating Alcaftadine was validated for the following parameters using ICH Q2(R1) guidelines. ⁹⁻¹⁵

Specificity

Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present. Blank, standard solution prepared and injected to check peak purity. The peak purity for blank and placebo showed no interference at R.T. of alcaftadine. The peak purity of standard solution and test solution were observed at 0.972 and 0.968 respectively. The peak purity of standard and test solution within the limit. Acceptance criteria for peak purity not less than 0.95. Hence the developed chromatographic method passed the criteria for specificity.

Linearity and Range

The relationship between Alcaftadine concentration and corresponding UV intensity was found to be linear over the concentration range of 0.5-7.5µg/mL with a r^2 of 0.99995.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

$\sigma = 28303.40$ (Residual standard deviation of a regression line)

$s = 981096.494$ (Slope)

Detection limit (LOD):

$$LOD = 3.3 \sigma / S$$

$$LOD = 3.3 \times 28303.40 / 981096.494$$

$$LOD = 0.095 \mu\text{g/mL}$$

Quantitation limit (LOQ):

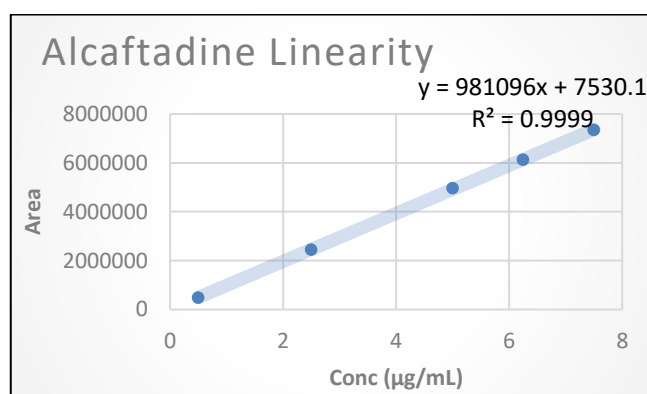
$$LOQ = 10 \sigma / S$$

$$LOQ = 10 \times 28303.40 / 981096.494$$

$$LOQ = 0.288 \mu\text{g/mL}$$

Accuracy (Recovery)

The accuracy of 50 %, 100% and 150 % was found to be 99.88, 100.64 and 100.11% respectively of 3 levels and % relative standard deviation was not more than 2.0%. % Recovery not get hampered by changed in analyte concentration.

**Figure 4:** Calibration curve of Alcaftadine**Precision**

Precision was performed on test sample. HPLC method was precise. The result shown in table 4.

Table 3: Result and statistical data of Accuracy of Alcaftadine

Level (%)	Alcaftadine Added Conc (µg/mL)	Area	% Recovery	Mean Recovery	% RSD
50	2.50	2431528	99.57	99.88	0.296
	2.50	2445760	100.16		
	2.50	2439457	99.90		
100	5.00	4935254	101.05	100.64	0.356
	5.00	4906085	100.45		
	5.00	4903840	100.41		
150	7.50	7302460	99.68	100.11	0.370
	7.50	7348206	100.30		
	7.50	7350518	100.34		

Table 4. Precision data for Alcaftadine test sample

Sr. No	Parameters	Intraday Precision	Interday Precision
1	Mean	99.70	100.08
2	SD	0.8160	0.3303
3	%RSD	0.818	0.330

Robustness

Following changes in wavelength, flow rate and column oven temperature made under Robustness. From the results shown in table 5, it was concluded that the system suitability test result was found within the limits and analytical method was robust.



Table 5: Result of Robustness study

Sr. no.	Parameter	Observations						Limit
		Changes in flow Rate (mL/min)		Change in Wavelength (nm)		Change in Column Oven temperature		
		1.1	0.9	285	279	42 °C	38 °C	
1	Theoretical Plate	7105	8394	7694	7707	7651	7456	NMT 2000
2	Peak area response	4466466	5464911	4488441	5080780	4930475	4820347	
3	Tailing factor	1.17	1.13	1.14	1.15	1.16	1.17	NMT 2.0
4	R.T.(Min)	2.77	3.39	3.05	3.05	3.05	3.05	

CONCLUSION

The aim of this project was to create a simple, reliable, precise, and appropriate RP-HPLC system. The established method of analysis results was validated in terms of linearity, accuracy, precision and robustness as well as the detection and quantification limits.

The developed method has many advantages, including reproducibility of findings, rapid interpretation, easy sample preparation and improved selectivity and sensitivity. In present study, the retention time was less than previous method reported.

The developed method can be used for routine research in the pharmaceutical industry for the bulk drug Alcaftadine as well as the pharmaceutical dosage type since it is stable and reproducible and takes less time.

According to the above experimental results, this newly developed method for estimating Alcaftadine was found to be simple, precise and accurate with a shorter retention time that makes it more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions quality control departments in industries and approved testing laboratories.

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