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Development and Validation of HPLC Method for the Determination of Alcaftadine in Bulk Drug and its Ophthalmic Solution

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Abstract

A simple, precise and accurate stability indicating RP-HPLC method was developed for the estimation of Alcaftadine in bulk drug and its ophthalmic dosage form. Chromatographic separation of target analyte and degradation products was achieved using Enable HPLC ODS C_{18} G (250 × 4.6 mm, 5 µm) column and mobile phase comprising of methanol: water, 50:50% v/v at a flow rate of 1.2 ml/min with UV detection at 282 nm. The correlation coefficient (r^2) was found to be 0.999 in the concentration range of 1-16 µg/ml. The retention time was found to be 3.15 min. The limit of detection and limit of quantitation was found to be 0.25 µg/ml and 0.75 µg/ml respectively. Stress degradation of Alcaftadine was carried out under stress conditions like acid and base hydrolysis, oxidation, thermal and photolytic stress. The degradation products generated as a result of stress did not show any interference to the detection of Alcaftadine. The developed method was validated, and selectivity of the method was demonstrated by its ability to quantify the target analyte in presence of its degradation products.

Keywords: Alcaftadine; Lastacaft; HPLC; Stability indicating method

Introduction

The IUPAC name of Alcaftadine is 6,11-dihydro-11-(1-methyl-4-piperidinylidene)-5H-imidazo [2, 1-b][3] benzazepine-3-carboxaldehyde (Figure 1). Alcaftadine is an ophthalmic dual-acting H_1-antihistamine and mast cell stabilizer approved for the prevention of itching associated with allergic conjunctivitis. The drug was approved by USFDA in July 2010. It is commercially marketed under the name LASTACAFT. Lastacaft is a sterile, topically administered H_1 receptor antagonist containing Alcaftadine for ophthalmic use [1-2].

Alcaftadine is not official in any Pharmacopoeia. A literature survey on Alcaftadine revealed that, until now no analytical method was reported for its determination in bulk drug and its ophthalmic dosage form. However, a clinical pharmacological review report found during the survey showed that liquid chromatography with tandem mass spectrometry (LC/MS/MS) was used to quantitate concentrations of Alcaftadine and R90692 (active metabolite) in K3 EDTA human plasma [3]. It was also found that the metabolic fate of 14 C- Alcaftadine was determined by high performance liquid chromatography-based separation of parent compound from metabolites [4]. Since literature did not cite any method for determination of this drug from bulk drug as well as its formulation it was planned to develop a RP-HPLC method to determine alcftadine in presence of its degradation products.

Materials and Methods

Instrumentation

Shimadzu Prominence UFLC LC-20AD with UV detector and Enable C_{18} G RP column (250 × 4.6 mm, 5 µm) was used was chromatographic resolution of target analyte and degradation products. The acquisition and integration of data was performed using LC solution software.

Materials and reagents

Alcaftadine pure drug sample was obtained from JHP Pharmaceuticals LLC, Rochester, Michigan, USA. Lastacaft, Alcaftadine ophthalmic solution, was procured from MediPrime Pharmacy, Dubai. HPLC grade methanol and HPLC water was procured from Merck.

Solution preparation

Standard stock solution: Accurately 10 mg of drug was weighed and transferred to a 10 ml volumetric flask. It was dissolved using methanol and volume was made up to obtain a concentration of 1000 µg/ml. After sufficient dilutions, final stock having concentration equivalent to about 50 µg/ml of drug was prepared.

Working standard solution: The standard stock solution was used to prepare working standard solutions of concentrations 1, 4, 8, 12 and 16 µg/ml. Solution having drug concentration of 8 µg/ml was used as a working standard for stress degradation studies. Both the standard and sample solution of 8 µg/ml were estimated at 282 nm and the chromatograms were recorded (Figure 2).

Sample solution

From the ophthalmic solution (alcaftadine 2.5 mg/ml), one ml

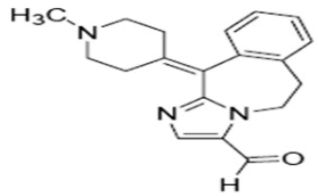

Figure 1: Structure of alcaftadine.

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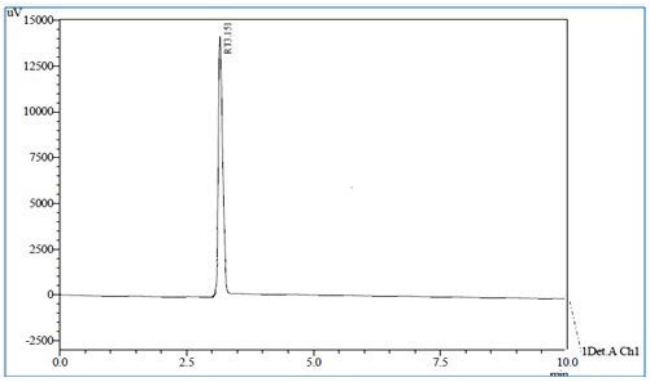

Figure 2: Chromatogram of alcaftadine.

was withdrawn and transferred to a 10 ml volumetric flask, dissolved and diluted with methanol to get the concentration of 2500 µg/ml. Further dilutions were made to get the final stock having concentration equivalent to 8 µg/ml.

Optimization of chromatographic conditions

Various trials were made for the selection of chromatographic conditions like mobile phase, its ratio and flow rate. Finally, the one giving the best results were optimized. The chromatographic estimation of Alcaftadine and its separation from degradation products was achieved using Enable C_{18} G HPLC column using mobile phase Methanol: Water 50:50% v/v at a flow rate of 1.2 ml/min. The UV detection was done at 282 nm. The optimized chromatographic conditions are summarized in Table 1.

Validation of proposed method

The method was validated for the parameters like specificity, linearity, precision, accuracy and robustness as per ICH guidelines [5,6].

System suitability testing

Six replicates of drug concentration of 8 µg/ml were injected and the chromatograms were recorded to check with the system suitability parameters [7].

Forced degradation studies

The forced degradation studies were carried out as per ICH Q1A (R2) for hydrolysis, oxidation and thermal stress conditions and ICH Q1 B for photo stability. The stress conditions employed were 1N HCl for acid hydrolysis, 1 N NaOH for base hydrolysis, 3% H_2O_2 for oxidative hydrolysis. The drug samples were also subjected to thermal stress conditions as well as subjected to UV light to test photo stability [8-15].

Acid and base hydrolysis: Acid and base induced forced degradation was performed by adding an aliquot of stock solution (1 mg/ml) of Alcaftadine to 1 N HCl and 1N NaOH respectively. These solutions were subjected to stress condition of 50°C for 24 hours. The resulting solutions were sufficiently diluted to obtain the concentration of 8 µg/ml.

Oxidation stress: To study the effects of oxidative conditions, aliquot of stock solution (1 mg/ml) was added to 3% H_2O_2 solution. It was then kept at 50°C for 24 hours. The resulting solution was sufficiently diluted to obtain the concentration of 8 µg/ml.

Thermal stress: The effect of temperature on Alcaftadine solution was studied by subjecting aliquot of stock solution (1 mg/ml) to hot air oven at 80°C temperature for two hours. The final solution of 8 µg/ml was prepared by adequate dilutions.

Photolytic stress: The aliquot of standard stock solution (1 mg/ml) was subjected to the UV exposure in UV chamber for 30 minutes. The resulting solution was adequately diluted to obtain the final concentration of 8 µg/ml.

Results and Discussion

Selection of solvent

Since Alcaftadine was soluble in methanol, it was used as a solvent. For dilution purpose also methanol was only used because on further dilutions with water resulted in splitting of peak.

Optimization of mobile phase

Final mobile phase Methanol: water was selected since trials done using ACN: Water caused peak splitting with different ratios. The ratio 50:50% v/v was optimized because altering the ratio to 60:40 or 70:30 resulted in asymmetrical peak. The flow rate 1.2 ml/min was optimized since at lower flow rates, i.e., at 0.8 ml/min or 1 ml/min, peak tailing of more than 2 was observed.

Optimization of chromatographic conditions

The aim was to develop a method so that it can specifically identify the analyte peak in presence of its degradants. The chromatographic separation of Alcaftadine from its degradants was achieved using Enable C_{18} G RP HPLC column with mobile phase Methanol: Water (50:50% v/v) at a flow rate of 1.2 ml/min and detection wavelength of 282 nm.

System suitability testing

The results of the system suitability parameters are shown in Table 2. The results were found within the acceptable limits. Hence the system was suitable for the proposed method.

Validation

Linearity: The linearity was observed in the concentration range of 1-16 µg/ml for Alcaftadine. The regression line equation was plotted between concentration and peak area. The regression equation y=1639x+50431 was obtained from the linearity data. The correlation coefficient was found to be 0.999.

Chromatographic parameters	Optimized conditions
Stationary phase	Enable HPLC C18 G 120A Column 250 × 4.6 mm, 5 µ
Mobile phase	Methanol: water, 50:50 (%v/v)
Flow rate	1.2 ml/min
Injection volume	20 µl
Detection wavelength	282 nm
Run time	10 minutes

Table 1: Optimized Chromatographic Conditions for RP-HPLC.

S No	Parameters	Observed results (n=6)	Acceptance criteria	Remarks
1	Theoretical plates (N)	3088.474	>2000	Method passes the system suitability test
2	Tailing factor (T)	1.36	T ≤ 1.5	
3	Repeatability (% RSD)	0.28	%RSD<2	
4	Capacity factor (k')	2.58	K'>2	

Table 2: Results for System Suitability Test by RP-HPLC.

Specificity: The results of specificity are shown in Figure 3. It showed no interference of the excipients present in drug formulation.

Precision: The results of precision studies are summarized in Table 3. The % RSD was found within the acceptable limit, i.e., <2.

Recovery studies: The % recovery was observed within the acceptable limits, i.e., 98.71%, 99.06% and 99.39% at the levels of 80%, 100% and 120% respectively. The results are summarized in Table 3.

LOD and LOQ: The results of LOD and LOQ were found to be 0.25 µg/ml and 0.75 µg/ml respectively. The results are summarized in Table 3.

Robustness: Robustness of the method was studied by making variations in the parameters like flow rate (± 0.2 ml/min), mobile phase composition (± 0.2) and detection wavelength (± 2 nm). The deliberate changes made in the flow rate, mobile phase composition and wavelength did not show major impact on retention time, assay value and peak area.

Forced degradation studies

Both the drug (API) as well as formulation was subjected to various stress conditions like acid/ base hydrolysis, oxidation, thermal and photolytic stress conditions as per ICH guidelines. The results are tabulated in Table 4.

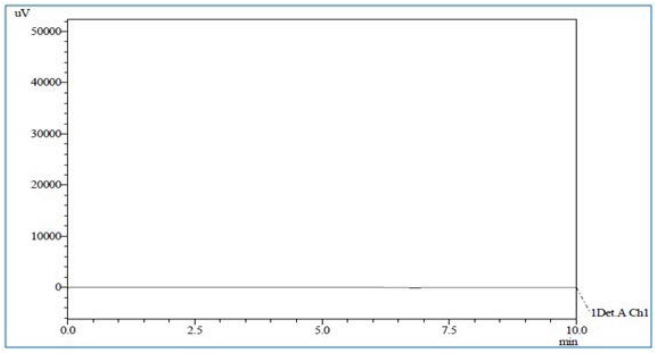

Figure 3: Blank chromatogram of mobile phase.

Parameters	Results	
Calibration curve range	1-16 µg/ml	
Regression line equation	Y=1639x+50431	
Slope	1639	
Intercept	50431	
Correlation coefficient (r^2)	0.999	
Summary of Validation		
Accuracy	**% Recovery**	**% RSD**
80%	98.87	0.495
100%	98.95	1.340
120%	99.46	0.330
Precision	**Concentration (µg/ml)**	**% RSD**
Repeatability (n=6)	8	1.205
Intraday precision (n=3)	4	0.986
	8	0.920
	12	1.08
Interday precision (n=3)	4	0.982
	8	0.582
	12	0.780
LOD	0.25 µg/ml	
LOQ	0.75 µg/ml	
Robustness	No significant changes observed with deliberate changes in the method parameters	

Table 3: Summary of the developed and validated HPLC Method.

Stress conditions	% Degradation	
	API	Formulation
Acid hydrolysis (1N HCl) 50°C, 24 hours	14.88%	14.32%
Base hydrolysis (1N NaOH) 50°C, 24 hours	15.23%	14.28%
Oxidation (3% H_2O_2) 50°C, 24 hours	6.89%	6.35%
Thermal (80°C, 2 hours)	15.13%	16.52%
Photostability (UV chamber, 30 mins)	26.32%	24.02%

Table 4: Results of Forced Degradation Studies.

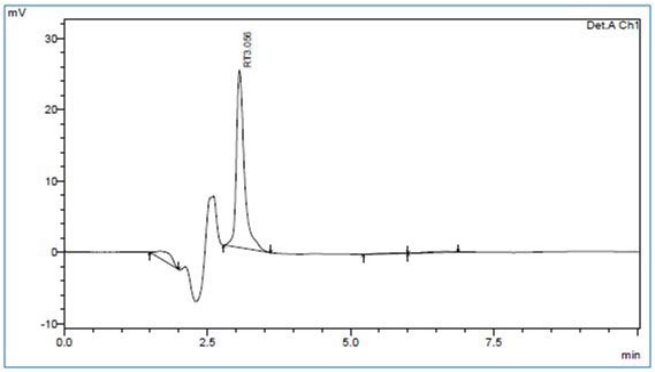

Figure 4: Chromatogram of acid hydrolyzed Alcaftadine.

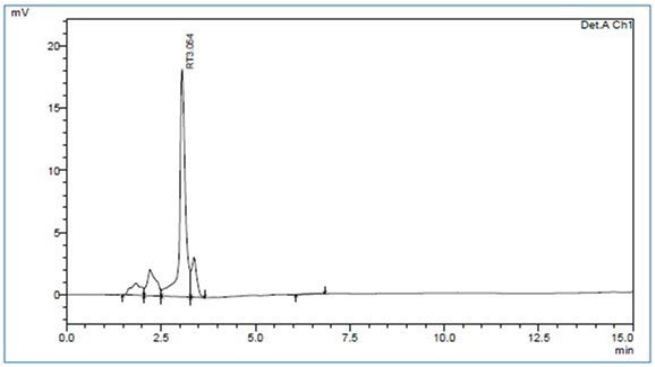

Figure 5: Chromatogram of base hydrolyzed Alcaftadine.

Acid and base hydrolysis: The drug degraded in the applied stress conditions of the acid and base hydrolysis i.e., 14.88% and 15.23% respectively for API and 14.32% and 14.28% respectively for formulation. The chromatograms showed the presence of degraded products (Figures 4 and 5).

Oxidation degradation: The drug was found to be quite stable in oxidation stress condition. It showed 6.89% and 6.35% for API and formulation respectively (Figure 6).

Thermal degradation: Adequate thermal degradation was achieved under thermal stress i.e., 15.13% and 16.52% for API and formulation respectively (Figure 7).

Photolytic degradation: The rate of photolytic degradation was higher as compared to others. The drug was degraded sufficiently i.e., 26.32% and 24.02% for API and formulation respectively (Figure 8).

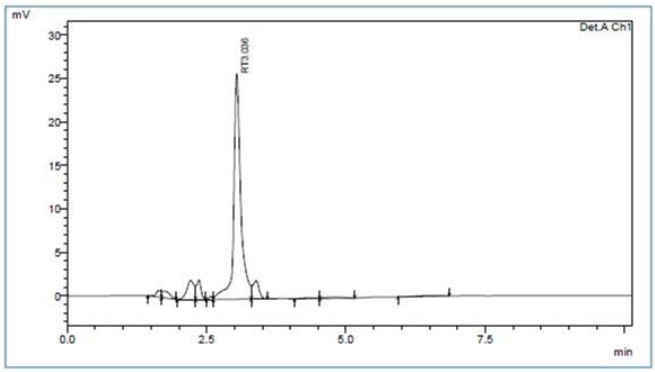

Figure 6: Chromatogram of Alcaftadine under oxidation.

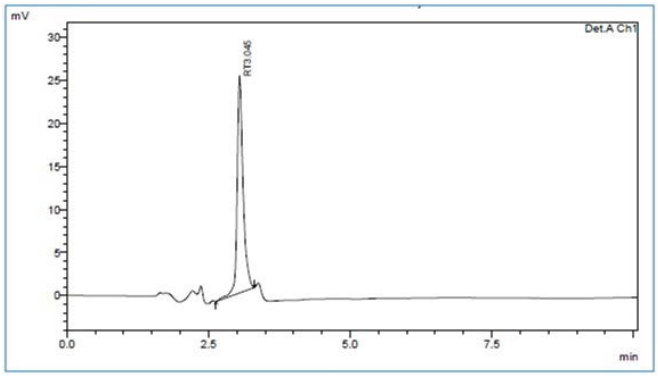

Figure 7: Chromatogram of thermally degraded Alcaftadine.

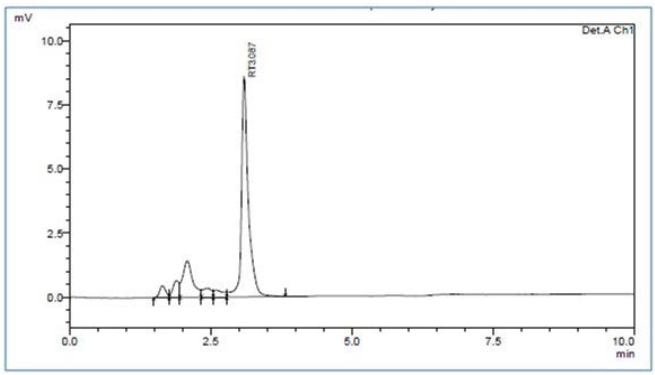

Figure 8: Chromatogram of Alcaftadine during photostability studies.

Formulation	Labeled amount ml/ml	Amount found mg/ml	% Label claim ± SD
LASTACAFT (ophthalmic solution)	2.5	2.49	99.90 ± 0.698

Table 5: Results for the Assay of Ophthalmic Solution of Alcaftadine.

Analysis of the marketed formulation

The proposed method was applied to the marketed formulation of Alcaftadine (LASTACAFT ophthalmic solution, 2.5 mg/ml). The assay results were found to be within acceptable limits, i.e., 99.10-100.39%. The results are shown in Table 5.

The developed RP-HPLC method for determination of alcaftadine from ophthalmic preparations is specific, reliable and accurate. The observed results also showed the value of relative standard deviations below 2, which indicate the accuracy and precision of the developed method. The proposed method is capable of identifying the target analyte in presence of its degradants. The degradation products generated during stress conditions were not further characterized.

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References

1. National PBM Drug Monograph (2015) Alcaftadine 0.25% Ophthalmic Solution (Lastacaft). VA Pharmacy Benefits Management Services. Medical Advisory Panel and VISN Pharmacist Executives.
2. (2015) US Food and Drug Administration Protecting and Promoting Your Health.
3. (2015) Office of Clinical Pharmacology Review.
4. Bohets H, McGowan C, Mannens G, Schroeder N, Edwards-Swanson K, et al. (2011) Clinical pharmacology of alcaftadine, a novel antihistamine for the prevention of allergic conjunctivitis. J Ocul Pharmacol Ther 27: 187-195.
5. ICH, Q2A, Hamonised Tripartite Guideline, Test on Validation of Analytical Procedures, IFPMA. In: Proceedings of the International Conference on Harmonization, Geneva. March, 1994.
6. ICH, Q2B, Hamonised Tripartite Guideline, Validation of Analytical Procedure: Methodology. IFPMA, In: Proceedings of the International Conference on Harmonization, Geneva. March, 1996.
7. Nash AR, Wachter AH (2005) Pharmaceutical process validation. 3rd edn. New York: Marcel Dekker Inc.
8. ICH, Q1A (R2): Stability Testing of New Drug Substances and Products (Revision 2), International Conference on Harmonization.
9. ICH, Q1B: Photostability Testing of New Drug Substances and Product, International Conference on Harmonization.
10. Biradar SP, Kalyankar TM, Wadher SJ, Moon RS, Dange SS (2014) Stability indicating HPLC method development: Review. Asian J Med and Anal Chem 1: 21-26.
11. Ngwa G (2010) Forced degradation studies as an integral part of HPLC stability indicating method development. Drug Deliv Technol 10: 56-59.
12. Blessy M, Patel RD, Prajapati PN, Agrawal YK (2014) Development of forced degradation and stability indicating studies of drugs- A review. J Pharm Anal 4: 159-165.
13. Bakshi M, Singh S (2002) Development of validated stability-indicating assay methods--critical review. J Pharm Biomed Anal 28: 1011-1040.
14. Singh S, Bakshi M (2000) Guidance on conduct of stress tests to determine inherent stability of drugs. Pharm Tech online 24: 1-14.
15. Brummer H (2011) How to approach a forced degradation study. Life Sci Technol Bull 31: 1-4.