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# DEVELOPMENT AND VALIDATION OF UV- SPECTROPHOTOMETRY AND THE FIRST ORDER DERIVATIVE USING THE AREA UNDER CURVE METHOD FOR THE ESTIMATION OF ALCAFTADINE IN BULK AND ITS OPHTHALMIC DOSAGE FORM

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## ABSTRACT

The present work aims to develop and validate two simple UV spectrophotometric methods including area under curve method of zero order and first-order derivative respectively for the estimation of Alcaftadine in bulk drug and its application on marketed formulation. In this study, methanol was used as a solvent. In AUC of zero order derivative method (METHOD A), two wavelengths 266 nm and 296 nm and in AUC of first order derivative method (METHOD B), two wavelengths 257 nm and 277 nm were selected for the determination of integrated areas. Linearity was found in the concentration range of 1-16 µg/ml for both the methods and correlation coefficient was found to be 0.999 and 0.998 for methods A and B respectively. The proposed methods were found to be simple, precise and accurate and can be employed for routine quality control analysis of Alcaftadine in bulk drug as well as ophthalmic dosage forms.

**KEYWORDS:** Alcaftadine, UV Spectrophotometry, Area under Curve, First Derivative

## INTRODUCTION

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed, scattered or emitted by atoms, molecules, or other chemical species. This absorption or emission is associated with changes in the energy states of the interacting chemical species and, since each species has characteristic energy states, spectroscopy can be used to identify the interacting species [1].

Chemically Alcaftadine is 6, 11-dihydro-11-(1-methyl-4-piperidinylidene)-5H-imidazo [2, 1-b] [3] benzazepine-3-carboxaldehyde (Figure 1). The drug product, Alcaftadine, 2.5 mg/ml ophthalmic solution, is an isotonic solution for a once daily dosing regimen in the prevention of itching associated with allergic conjunctivitis. Alcaftadine is an ophthalmic dual-acting $H_1$-antihistamine and mast cell stabilizer approved for the prevention of itching associated with allergic conjunctivitis. The dose is 1 drop in each eye once daily. 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 in July 2010. It is commercially marketed under the name LASTACAFT [2 – 4]. Alcaftadine is not official in BP, IP and USP. A literature survey on Alcaftadine revealed that no proposed analytical method was reported for estimation of Alcaftadine in bulk drug and its ophthalmic dosage form.

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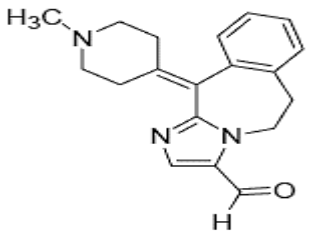
**Figure 1.** Structure of Alcaftadine




However clinical pharmacological review report was found during the survey in which 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 [4]. Also, metabolic fate of 14 C- Alcaftadine was determined by high performance liquid chromatography-based separation of parent compound from metabolites [5].

The present work aims at developing simple, precise and accurate UV- spectrophotometric and first-order derivative methods for routine estimation of Alcaftadine in bulk drug and its application to the ophthalmic solution. The analytical method for Alcaftadine is not officially available in any pharmacopoeia. Therefore an attempt was made to develop analytical spectroscopic methods which can be employed for routine analysis of Alcaftadine. The developed method was validated for linearity, accuracy, and precision, limit of detection and limit of quantification.

## MATERIALS AND METHODS

### Instruments

1. Digital precision balance (ATX 224),
2. UV-visible spectrophotometer (SHIMADZU-1800).

### Reference Standard

Alcaftadine API was procured from JHP Pharmaceuticals, LLC.

| Trade name | Company name | Dose | Batch number | Manufactured date | Expiry date |
|---|---|---|---|---|---|
| LASTACAFT | ALLERGAN | 2.5mg/mlL | 01744 | April 2014 | March 2016 |

**Figure 2.** AUC of zero-order spectrum of Alcaftadine in the range 266 – 296 nm

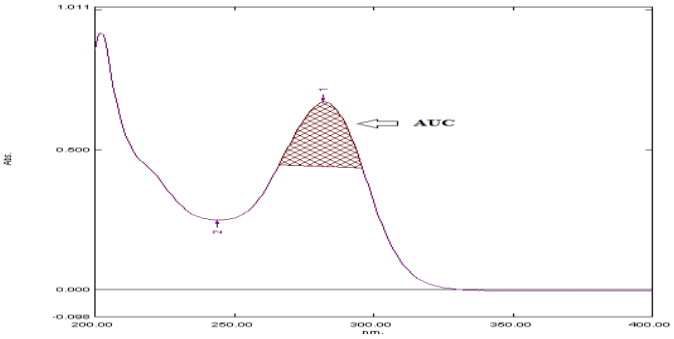



**Figure 3.** AUC of first order derivative spectrum of Alcaftadine in the range 257 – 277 nm

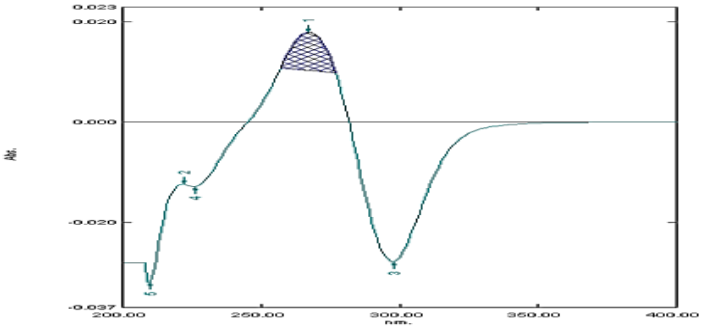


**Table 1.** Linearity data of Alcaftadine

| Concentration (µg/ml) | AUC of zero order spectra | AUC of First order derivative |
|---|---|---|
| 1 | 0.5350±0.003 | 0.012±0.0 |
| 4 | 2.1613±0.006028 | 0.049±0.0 |
| 8 | 4.3496±0.01204 | 0.0983±0.001155 |
| 12 | 6.4870±0.020664 | 0.147±0.000577 |
| 16 | 8.4320±0.01159 | 0.191±0.0 |
| Regression equation | Y=0.529x+0.052 | Y=0.012x+0.001 |
| Correlation coefficient | 0.999 | 0.999 |

## Marketed Formulation

The marketed formulation of Alcaftadine was procured from MediPrime Pharmacy, Dubai.

## Methods

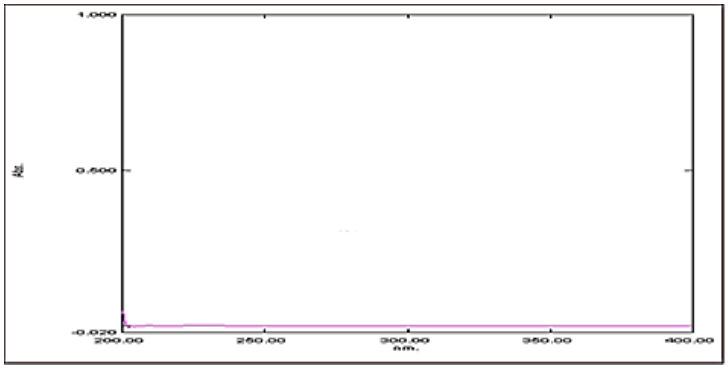
### *Preparation of standard stock solution*

The standard stock solution was prepared by dissolving 10mg drug in methanol and diluting it with the same solvent up to 10ml to obtain a concentration of 1000 µg/ml. The stock solution was further diluted to give a solution with concentration, 50 µg/ml. After appropriate dilution, 10 µg/mL solution of Alcaftadine was scanned in the UV region, i.e. 400 – 200 nm. Alcaftadine showed maximum absorption $\lambda_{max}$ 282 nm in methanol.

**Table 2.** Precision studies of Alcaftadine

| Precision | Concentration (µg/ml) | % RSD | |
|---|---|---|---|
| | | Method A | Method B |
| **Repeatability (n = 6)** | 8 | 0.173 | 0.852 |
| **Intra-day precision (n = 3)** | 4 | 0.112 | 0.745 |
| | 8 | 0.020 | 0.742 |
| | 12 | 0.086 | 0.396 |
| **Inter-day precision (n = 3)** | 4 | 0.290 | 0.0 |
| | 8 | 0.270 | 0.573 |
| | 12 | 0.320 | 0.804 |

**Figure 4.** Blank spectra of placebo



### *Preparation of working standard solution*

The standard stock solution was suitably diluted with methanol to get working standard solutions of concentrations 1, 4, 8, 12, 16 µg/ml. These solutions were scanned in the wavelength range of 200 – 400 nm.

**Figure 5.** Calibration curve of AUC of zero order

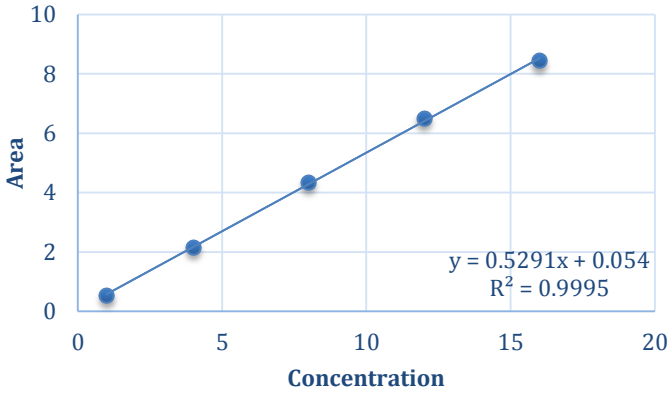


**Table 3.** Recovery studies: (n = 3)

| Recovery level (%) | Amount present (µg/ml) | Amount spiked (µg/ml) | Total amount of drug (µg/ml) | Methods | Total Amount recovered (µg/ml) | Mean % recovery | % RSD |
|---|---|---|---|---|---|---|---|
| **80** | 5 | 1.4 | 6.4 | **A** | 6.46 | 104.32 | 0.675 |
| | | | | **B** | 6.42 | 100.85 | 0.012 |
| **100** | 5 | 3 | 8.0 | **A** | 8.03 | 100.90 | 0.329 |
| | | | | **B** | 8.0 | 99.73 | 0.370 |
| **120** | 5 | 4.6 | 9.6 | **A** | 9.71 | 102.22 | 0.421 |
| | | | | **B** | 9.66 | 101.27 | 0.019 |

**Figure 6.** Calibration curve of AUC of first order derivative

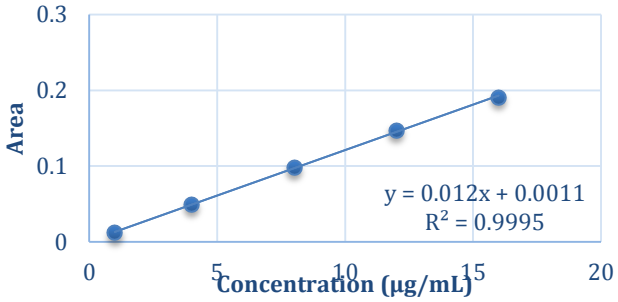



**Table 4.** Assay of marketed formulation

| Formulation | Eye drops LASTACAFT | |
|---|---|---|
| Method | A | B |
| **Labeled amount (mg/ml)** | 2.5 | 2.5 |
| **Amount found (mg/ml)** | 2.54 | 2.54 |
| **% Label claim ± SD Assay (n = 3)** | **101.77±0.410** | **101.60±0.911** |

***Preparation of sample solution:***

From the ophthalmic solution containing 2.5 mg/ml of Alcaftadine, 1 ml was withdrawn and transferred into a 10 ml volumetric flask and diluted with methanol to make a stock of 2500 µg/ml. The final stock of 50 µg/ml was prepared by making appropriate dilutions with the methanol.

***Area under curve method [6, 7]***

The area under curve method is used when a broad spectrum of the drug is obtained. From the spectrum of Alcaftadine, the area under the curve in the range of 266 – 296nm for the zero-order spectra (Figure 2) and 257 – 277nm for the first order derivative spectra were selected for the determination of areas (Figure 3).

**Validation of the proposed methods [8, 9]**

***Specificity***

The specificity of the method was determined by checking the interference of placebo with analyte. To evaluate interference from blank (mobile phase), or impurities present in the drug matrix, specificity was performed to identify the specific absorbance of the drug.

**Table 5.** Spectrophotometric characteristics of Alcaftadine

| Parameters | AUC of zero order spectra | AUC of First order derivative |
|---|---|---|
| **Wavelength** | 266 – 296 nm | 257 – 277 nm |
| **Beer's range** | 1-16 µg/ml | 1-16 µg/ml |
| **Regression equation** | Y = 0.529x + 0.052 | Y = 0.012x + 0.001 |
| **Correlation coefficient** | 0.999 | 0.999 |
| **Intercept** | 0.052 | 0.001 |
| **Slope** | 0.529 | 0.012 |
| **Sandell's Sensitivity (µg/cm²/0.001 absorbance unit)** | 0.09 | 0.010 |
| **Limit of detection (µg/ml)** | 0.187 | 0.317 |
| **Limit of Quantitation (µg/ml)** | 0.555 | 0.962 |

***Linearity studies***

From the standard stock solutions of Alcaftadine (50 µg/ml), 0.2 – 3.2ml were accurately transferred into five separate volumetric flasks and the volume was made up with methanol to obtain the concentration range of 1 – 16 µg/ml. The area was measured at selected wavelengths, and calibration curves were plotted as concentration versus area.

***Precision***

Repeatability was carried out for the working standard (8 µg/ml) for six replicates and % RSD was calculated. Inter-day and intraday precision was evaluated by estimating response of three replicates at three different concentrations i.e. 4, 8 and 12 µg/ml, on three different days and on the same day.

***Recovery studies***

The accuracy of the method was studied at three different levels, i.e. 80, 100 and 120 % levels. To the pre-analyzed sample solution (5 µg/mL of Alcaftadine), a known amount of drug standards of Alcaftadine was added. The percent recovery was estimated by the proposed method.

**Table 6.** Summary of validation parameters

| Parameters | Results | | | |
|---|---|---|---|---|
| | Method A | | Method B | |
| **Linearity (µg/ml)** | 1 – 16 (µg/ml) | | 1 – 16 (µg/ml) | |
| **Precision** | % RSD | | | |
| **Repeatability (n = 6)** | 0.276 | | 0.852 | |
| **Interday precision (n = 3)** | 0.276 – 0.323 | | 0.0 – 0.804 | |
| **Intraday precision (n = 3)** | 0.275 – 0.323 | | 0.396 – 0.751 | |
| **Accuracy (n = 3)** | % recovery | | %RSD | |
| | Method A | Method B | Method A | Method B |
| **80%** | 104.32 | 100.85 | 0.675 | 0.012 |
| **100%** | 100.90 | 99.73 | 0.329 | 0.370 |
| **120%** | 102.22 | 101.27 | 0.421 | 0.019 |

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

LOD and LOQ were calculated by application of following formula;

$$LOD = 3.3\sigma / S$$
$$LOQ = 10\ \sigma / S$$

where, σ = standard deviation of response; S = slope of calibration curve

## RESULTS AND DISCUSSION

### Specificity

In order to evaluate interference from matrix components or impurities, specificity was performed. The blank sample i.e. placebo did not show any interference (Figure 4).

### Linearity

The absorbance were found to have a direct relationship with the concentration of the drug. The correlation coefficient ($r^2$) for method A was found to be 0.99 (Figure 5) and for method B, it was found to be 0.99 (Figure 6). The regression line equation was calculated by plotting a graph of concentration vs. AUC of zero order derivative for method A and concentration Vs AUC of first order derivative for method B. The linearity data are presented in Table 1.

### Precision

#### *Repeatability*

Repeatability was evaluated by analyzing six replicates of the standard drug of 8 µg/ml. % RSD was calculated.

#### *Intra-day precision*

Intraday precision was evaluated by estimating response of three replicates each for three different concentrations i.e. 4, 8 and 12 µg/ml on the same day by both the methods.

#### *Inter-day precision*

Intraday precision was evaluated by estimating response of three replicates each for three different concentrations i.e. 4, 8 and 12 µg/ml on the different days by both the methods. The results of precision studies are summarized in Table 2.

#### *Accuracy (Recovery studies)*

Alcaftadine % recovery of was found to be 100.90 – 104.32 % for method A and 99.73 – 101.27 % for method B. The results are shown in Table 3.

#### *Analysis of the marketed formulation*

The assay was carried out for the estimation of Alcaftadine in the marketed formulation by the proposed methods. The results of the assay were found to be reliable and hence proved the efficacy of the methods. The results are summarized in Table 4.

#### *Limit of Detection and Limit of Quantitation*

The results of LOD and LOQ are summarized in Table 5. The spectrophotometric characteristics of Alcaftadine and results of validation parameters are summarized in Table 5 and Table 6 respectively.

## CONCLUSION

The developed UV spectrophotometric methods were found to be simple, linear, precise and accurate. These methods can be employed for routine analysis of Alcaftadine in bulk and in ophthalmic dosage form.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