RESEARCH ARTICLE

Development and Validation of New UV Spectrophotometric Methods for Simultaneous Estimation of Alcaftadine in Combination with Ketorolac Tromethamine in a Pharmaceutical Dosage Form and Statistical Analysis using Analysis of Variance

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ABSTRACT

Alcaftadine and Ketorolac tromethamine combination is newly introduced in the market for the treatment of conjunctivitis. Hence, it is essential to develop a rapid, accurate, sensitive and precise method (in accordance with ICH guidelines) to estimate drugs. Three simple and cost-effective UV-spectrophotometric methods (First-order derivative (A), Dual Wavelength (B) and Q- absorbance ratio method (C) have been developed for simultaneous estimation of Alcaftadine and Ketorolac tromethamine in their pharmaceutical dosage form and statistical comparison using analysis of variance (ANOVA). Method (A) is based on the first-order derivative spectrophotometric method, in which zero-crossing points (ZCP) for Alcaftadine is 281.50 nm and Ketorolac tromethamine is 268 nm. Linearity was found in the 4–14 µg/mL range for Alcaftadine and 6.4–22.4 µg/mL for Ketorolac tromethamine using methanol as a common solvent. Method (B) is based on the principle of the dual-wavelength method using absorbance difference at 336 and 299.50 nm for Alcaftadine; 268 and 293.50 nm for Ketorolac tromethamine. Method (C) is constructed on Q-absorbance ratio method where the iso-absorptive point was obtained at 296 nm and the λ_{max} selected was of Alcaftadine 282 nm. The accuracy, precision, Limit of Detection (LOD), and Limit of Quantification (LOQ) of methods were determined and validated. All the developed methods showed good reproducibility and recovery with % RSD <2. These three methods developed were compared using the statistical method one-way ANOVA, and the f_{cal} value was found to be less than f_{tab} value, indicating that there is no significant difference in the assay results of the three methods. All three methods were found to be rapid, specific, precise, and accurate and found no interferences from the excipients, so it can be used for routine investigation of both drugs in quality control laboratories.

Keywords: Alcaftadine, ANOVA, Dual-wavelength, First-order derivative, Ketorolac tromethamine, Q-Absorbance, Validation.

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INTRODUCTION

Alcaftadine (ALC) is used to prevent itching in the eyes caused by allergies.[1-3] It belongs to the class of drug is Benzazepines.[4] Chemically is known as 11-(1-methylpiperidin-4-ylidene)-5,6-dihydroimidazo[2,1-b] [3] benzazepine-3-carbaldehyde (Figure 1). ALC is a broad-spectrum antihistamine displaying a high affinity for histamine H1 and H2 receptors and a lower affinity for H4 receptors.[4] Ketorolac Tromethamine (KTC) is **a** non-steroidal anti-inflammatory drug (NSAID) in the heterocyclic acetic acid derivative family, frequently used as an analgesic, antipyretic and anti-inflammatory.[5] Chemically, it is known as 5-benzoyl-2, 3-dihydro-1H-pyrrolizine-1carboxylic acid with 2-amino-2-(hydroxymethyl) propane-1,3-diol (Figure 2).

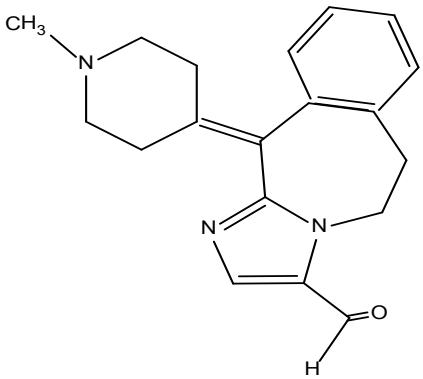

Figure 1: Chemical structure of ALC

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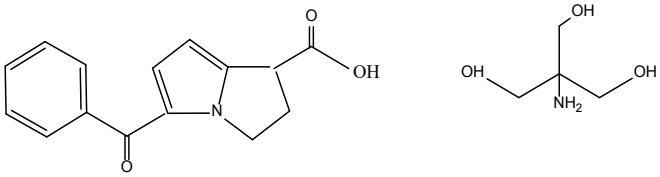

Figure 2: Chemical structure of KTC

The deep literature survey revealed that various spectrophotometric and chromatographic methods are available to estimate ALC[6-9] and KTC[10-17] alone or with some other drugs. Literature survey also showed that no reported method was available for the analysis of both drugs in a pharmaceutical dosage form. Due to the pharmaceutical importance of ALC and KTC combinations, this work concerns developing and validating three simple, sensitive, and selective spectrophotometric methods to determine the proposed drugs in their pure and pharmaceutical dosage forms. The most significant feature of this method is its simplicity, rapidity, and non-requiring time-consuming sample preparations such as extraction of solvents, derivatization, heating, and degassing, which are needed for the chromatographic procedure. This research aimed to develop and validate both drugs concurrently by simple, accurate, rapid, and precise first derivative spectrophotometric, dual-wavelength, and Q-absorbance assays for routine analysis.

MATERIALS AND METHODS

Materials

Reagents and Chemicals

ALC gift sample was provided by Apicore Pharma, Dabhasa, Vadodara, Gujarat. KTC gift sample was provided by MSN laboratories private Ltd. Hyderabad, India. Marketed formulation (Eye drops) containing 0.25% w/v of ALC and 0.4% w/v of KTC were purchased from local pharmacies. Pioneer Pharmacy Degree College, Vadodara, Gujarat, India provided reagents, glassware, and instruments.

Instrumentation and Apparatus

A double beam UV-visible Spectrophotometer (UV-1800 Shimadzu, Japan) with software UV-probe 2.33, with a spectral slit width of 2 nm, wavelength accuracy of 0.5 nm, and a pair of 1cm matched quartz cuvettes were used to measure absorbance. All weights were taken on an electronic balance (ATX 224 Shimadzu, Japan). Volumetric flasks and pipettes of borosilicate glasses were used in the experiments.

Selection of Common Solvent

Selection of solvent was made based on an assessment of ALC and KTC in different solvents like methanol, water, chloroform etc. The analytical grade of methanol was selected as a common solvent for method development.

Preparation of Solutions

The standard stock solution of each drug was prepared by accurately weighing 10 mg of ALC, and 10 mg of KTC transferred into 100 mL separate volumetric flasks, dissolving with a small amount of methanol, and diluted up to the mark with methanol. This solution gave (100 µg/mL of each ALC and KTC).

METHODOLOGY

Calibration Curve of ALC and KTC

For first-order derivative (4–14 µg/mL for ALC and 6.4–22.4 µg/mL KTC), dual-wavelength (4–14 µg/mL for ALC and 6.4–22.4 µg/mL KTC) and Q-absorbance spectrophotometric method (2–12 µg/mL for ALC and 3.2–19.2 µg/mL KTC), accurate aliquots of ALC and KTC were transferred from its stock solution (100 µg/mL, each drug) into a series of 10 mL volumetric flask and diluted up to mark with methanol and mix well.

Method A: First-order Derivative Spectroscopic Method

The working solution of ALC 8 µg/mL and KTC 12.8 µg/mL were scanned in the 200–400 nm range, and methanol was kept as the reference solution. Data were recorded at an interval of 2 nm. The spectra recorded were converted first to fourth-order derivative using UV probe software, and these spectra were analyzed for Zero-crossing point (ZCP) of both the ALC and KTC, respectively. The ZCP on the first derivative spectra of one drug, the other drug shows considerable absorbance, and these two wavelengths can be employed to estimate ALC and KTC without any interference from another drug in eye drops. Hence, to determine ALC and KTC, two analytical wavelengths were selected 268 and 281.50 nm, respectively. These absorbances vs. concentrations were plotted in the quantitative mode to obtain the calibration curve from which, by extrapolating the value of absorbances of the sample solution, the concentration of the corresponding drugs was determined. Both the drugs obeyed Beer's law.

Spectrophotometric Conditions for First-order Derivative Method:

Measurement mode: Spectrum
Scan speed: Medium
Band width: 1 nm
Wavelength range: 200-400 nm
δλ: 10000
Scaling factor: 10
ZCP of ALC: 281.50 nm
ZCP of KTC: 268 nm

Method B: Dual Wavelength Method

The utility of a dual-wavelength data processing program is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. This is directly proportional to the concentration of the component of interest, independent of the interfering components. The pre-requisite for the dual-wavelength method is the selection of two such wavelengths where the interfering component shows the same absorbance, whereas the component of interest shows a significant difference in absorbance with concentration. Prepared the

working solution of the ALC and KTC was scanned UV between 200-400 nm. Overlain spectra of both the drugs were obtained from which two wavelengths for ALC and KTC were selected. For ALC two wavelengths that are 268 and 293.50 nm were selected. The absorbance difference of ALC was zero, but KTC and Mixture have shown some significant absorbance differences at 268 and 293.50 nm. For KTC, two wavelengths that are 336 and 299.50 nm was selected. The absorbance difference of KTC was zero but ALC and Mixture had shown some significant difference at 336 and 299.50 nm. The estimation of ALC was done by calculating the absorbance difference at 336 and 299.50 nm while estimation of KTC was done by calculating the absorbance difference at 268 and 293.50 nm.

Method C: Q- absorption Method

Q-absorbance ratio method uses the ratio of absorbance at two particular wavelengths: the isoabsorptive point and the λ-max of one of the two components. Solution of ALC (6 µg/mL) and KTC (9.6 µg/mL) were prepared and scanned in the range of 200–400 nm against methanol as blank. The overlain spectra were obtained to determine isoabsorptive point. The iso-absorptive point was observed at 296 nm and the other wavelength selected was 282 nm (λ_{max} of ALC) and the concentration of each component was calculated as per equation.

$$Cx = (Qm - Qy / Qx - Qy) \times A/a_1$$

$$Cy = (Qm - Qx / Qy - Qy) \times A/a_2$$

$$Qm = A2/A1$$

Whereas,

Cx and Cy = concentration of x and y respectively,

A = Absorbance of sample at iso-absorptive wavelength,

$a_1$and a_2= Absorptivity of x and y respectively at isoabsorptive point,

$$Qm = \frac{\text{absorbance of sample solution at } \lambda_{max} \text{ of one of the drug}}{\text{Absorbance of sample solution at iso-absorbtive point}}$$

$$Qx = \frac{\text{Absorbtive of x at } \lambda_{max} \text{ of one of the components}}{\text{Absobtivity of x at isoabsorbtive point}}$$

$$Qy = \frac{\text{Absorptivity of y at } \lambda_{max} \text{ of one of the components}}{\text{Absorbtivity of y at isoabsorbtive point}}$$

Analysis of ALC and KTC in Ear Drops

A volume of eye drops equivalent to 2.5, and 4 mg of ALC and KTC were diluted up to 10 mL with methanol. Further dilutions were made with methanol to reach the calibration range of each drug and the solutions were treated according to the procedure for linearity of UV spectrophotometric method. These solutions were scanned according to the wavelength selected in different methods. Absorbance obtained from three methods was put into their respective calibration curve equations, and concentration was obtained. From this obtained concentration, %label claim was found.

VALIDATION PARAMETERS

According to ICH guideline (Q2 R1), these three methods were validated.[18]

Accuracy

The suggested method has been applied to determine the different concentrations of ALC and KTC (80, 100, 120%) within their linearity ranges, and the concentrations were calculated from their corresponding regression equation. The percentage recoveries and SD for each drug were calculated. The validity of the proposed methods was further assessed by applying the standard addition techniques.

Precision/Repeatability

The precision of the proposed methods was checked by scanning solution (n=6) of ALC and KTC repeatedly, without changing the parameters and measuring their absorbance.

Intermediate precision

For the developed methods, Intraday and Interday precisions were measured in terms of %RSD. For intraday precision experiment was repeated for 3 different time intervals in a day whereas for interday precision experiments were repeated for 3 consecutive days. The results for both were reported in terms of %RSD.

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

LOD & LOQ for the methods were calculated by using formulas

3 X s/m for Limit of detection and
10X s/m for Limit of quantitation.

Whereas s= standard deviation of intercept (n=6) and m = slope of the conforming calibration curve.

Analysis of Variance (ANOVA)

This statistical tool is used to check the variation between the three developed methods used to simultaneously estimate ALC and KTC in Pharmaceutical dosage form ($p < 0.05$).

RESULT AND DISCUSSION

Method A: First-order Derivative Spectroscopic Method.

First-order spectra show more resolution than zero-order spectra in terms of zero-crossing points. Figure 3 shows

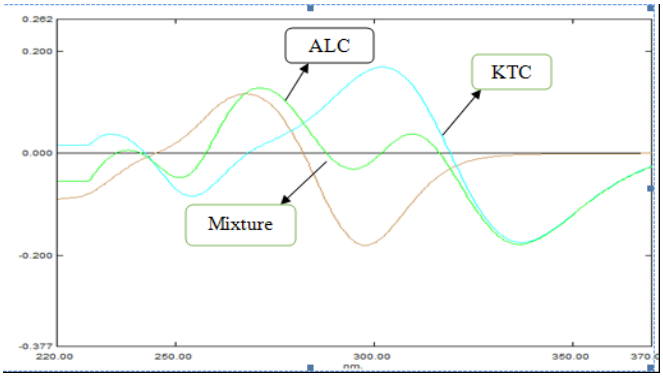

Figure 3: Overlain first-order derivative spectra of Alcaftadine (8 µg/mL) and Ketorolac tromethamine (12.8 µg/mL) and Mixture (8+12.8 µg/mL)

the overlain first-order spectra of ALC, KTC and mixtures, respectively. At 281.50 nm, ALC has a zero-crossing point and KTC can be estimated. At 268 nm, KTC has a zero-crossing point, and ALC can be estimated.

Method B: Dual-wavelength Method

Figure 4 shows the four selected wavelengths two for each drug where the drugs showed zero absorbance difference and their overlain spectra. Hence these wavelengths were used each other drugs by preparing calibration curves of absorbance difference for each drug.

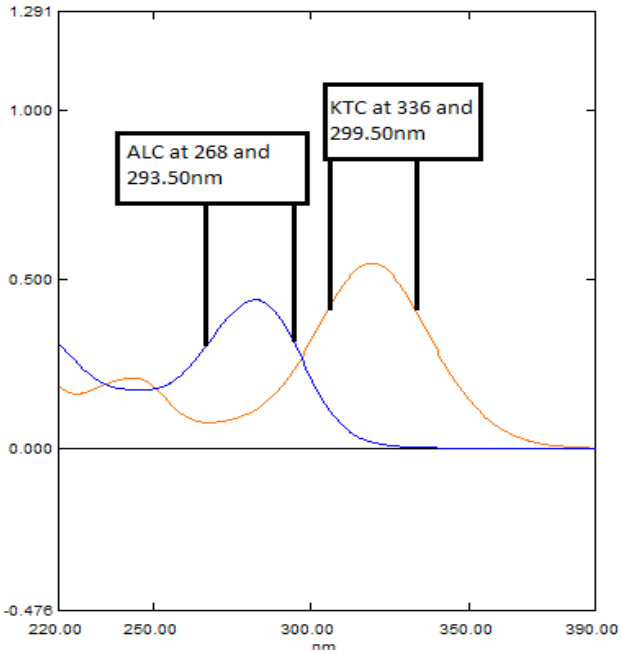

Figure 4: Overlain spectra of ALC (4 µg/mL) and KTC (6.4 µg/mL)

Method C: Q-Absorbance Method

Isoabsorptive point and the λ_{max} selected, along with their overlain spectra, were shown in Figure 5. 296 nm and were found to be an iso-absorptive point where both drugs showed the same absorbance. The calibration curves were plotted by taking absorbance for each drug on the selected λ_{max} and both drugs on iso-absorptive point.

Results of assay, accuracy studies, and summary of validation parameters are described in Table 1, Table 2, and Table 3, respectively.

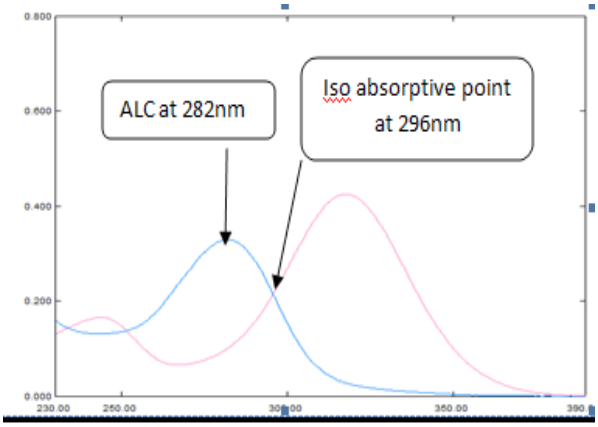

Figure 5: Overlain spectra of ALC (8 µg/mL) and KTC (12.8 µg/mL)

Table 1: Assay results for eye drop formulation using the proposed methods

		Label claim (mg)		*Amount of drug found (mg)*		*% Label claim Assay (n=6) ± SD*	
Formulation	*Method*	*ALC*	*KTC*	*ALC*	*KTC*	*ALC*	*KTC*
Eye drop	Method A	2.5	4	2.50	4.03	101.11 ± 0.605	100.84 ± 1.307
	Method B			2.51	4.0	100.51 ± 0.223	100.11 ± 1.002
	Method C			2.5&2.53	3.99&4.01	100.75 ± 0.001 & 101.5 ± 0.0007	99.84 ± 0.0005 &100.1 ± 0.002

Table 2: Application of the standard addition technique for recovery studies to the analysis of ALC and KTC in eye drops by the proposed methods

Method	*Drugs*	*Level (%)*	*Conc. Present (µg/mL)*	*Spiked Conc. (µg/mL)*	*Total Conc. taken (µg/mL)*	*Mean of total Conc. found (µg/mL)*	*Amt recovered (µg/mL)*	*%Recovery ± SD*	*%RSD*
Method A	ALC	80	6.0	4.8	10.8	10.91	4.82	100.48 ± 0.77	0.77
		100		6.0	12.0	12.14	6.05	100.84 ± 0.62	0.61
		120		7.2	13.2	13.28	7.19	99.88 ± 0.89	0.89
	KTC	80	9.6	7.7	17.3	17.26	7.68	99.24 ± 1.63	1.64
		100		9.6	19.2	19.16	9.58	99.0 ± 1.30	1.32
		120		11.52	21.12	21.21	11.63	100.18 ± 1.09	1.08

Method	*Drugs*	*Level (%)*	*Conc. Present (µg/mL)*	*Spiked Conc. (µg/mL)*	*Total Conc. taken (µg/mL)*	*Mean of total Conc. found (µg/mL)*	*Amt recovered (µg/mL)*	*%Recovery ± SD*	*%RSD*
Method B	ALC	80	6.0	4.8	10.8	10.80	4.80	100.15 ± 0.44	0.44
		100		6.0	12.0	11.95	5.96	99.37 ± 0.35	0.36
		120		7.2	13.2	13.16	7.16	99.55 ± 0.29	0.30
	KTC	80	9.6	7.7	17.3	17.27	7.66	99.57 ± 0.59	0.60
		100		9.6	19.2	19.19	9.58	99.00 ± 0.47	0.48
		120		11.52	21.12	21.03	11.42	99.37 ± 0.39	0.40
Method C	ALC	50	4.0	2	6	5.92	1.98	99.01 ± 0.84	0.85
		100		4	8	7.87	3.98	99.50 ± 0.42	0.42
		150		6	10	9.92	5.97	99.67 ± 0.28	0.28
	KTC	50	6.4	3.2	9.6	9.57	3.18	99.54 ± 1.12	1.13
		100		6.4	12.8	12.74	6.35	99.22 ± 0.97	0.98
		150		9.6	16	15.94	9.55	99.54 ± 0.37	0.37
	ALC and KTC At 296nm	50	4.0	2	6	5.90	1.96	99.39 ± 0.52	0.52
		100		4	8	7.87	3.96	99.09 ± 0.69	0.70
		150		6	10	9.90	5.96	99.49 ± 0.63	0.63
		50	6.4	3.2	9.6	9.55	3.18	99.60 ± 0.82	0.83
		100		6.4	12.8	12.74	6.37	99.66 ± 0.47	0.41
		150		9.6	16	15.91	9.55	99.50 ± 0.27	0.27

Table 3: Summary of validation parameters of developed methods

	Method A		*Method B*		*Method C*			
Parameters	*ALC*	*KTC*	*ALC*	*KTC*	*ALC*	*KTC*	*ALC*	*KTC*
Working wavelength (nm)	268	281.50	Absorbance difference at 336 &299.50 nm	Absorbance difference at 268 & 293.50 nm	282	282	296	296
Concentration range (µg/mL)	4–14	6.4–22.4	4–14	6.4–22.4	2–12	3.2–19.2	2–12	3.2–19.2
Regression equation	y = 0.0115x – 0.0066	y = 0.0046x + 0.002	y = 0.0268x – 0.0126	y = 0.0185x + 0.0017	y = 0.0553x – 0.0023	y = 0.0101x – 0.0032	y = 0.0347x – 0.0002	y = 0.0223x – 0.0019
Correlation coefficient (r2)	0.999	0.999	0.999	0.998	0.999	0.998	0.999	0.999
Mean of slope	0.015	0.004	0.026	0.012	0.055	0.010	0.034	0.022
SD of intercept	0.006	0.002	0.0006	0.0004	0.0004	0.002	0.004	0.013
LOD (µg/mL)	0.070	0.389	0.076	0.105	0.028	0.656	0.033	0.259
LOQ(µg/mL)	0.214	1.180	0.230	0.320	0.085	0.799	0.10	0.780
				Precision				
Repeatability (n=6) %RSD	1.75	1.22	0.31	0.78	0.66	0.77	0.79	1.46
Intraday(n=3) %RSD	0.279–1.075	0.490–1.88	0.09–0.22	0.54–0.75	0.10–0.22	0.34–0.82	0.16–0.47	0.16–0.45
Interday(n=3) %RSD	1.10.–1.19	0.490–1.88	0.49–1.11	0.67–1.47	0.10–0.22	0.34–0.82	0.16–0.48	0.54–0.66

Table 4: One way ANOVA of ALC

Source of variation	*SS*	*df*	*MS*	$F_{calculated}$	*P-Value*	$F_{tabulated}$
Between Groups	0.401111	2	0.200556	0.207997	0.814513	3.68232
Within Groups	14.46333	15	0.964222	-	-	-
Total	14.8644	17	-	-	-	-

Table 5: One way ANOVA of KTC

Source of variation	*SS*	*df*	*MS*	$F_{calculated}$	*P-Value*	$F_{tabulated}$
Between Groups	0.001078	2	0.000539	0.000616	0.999385	3.68232
Within Groups	13.12922	15	0.875281	-	-	-
Total	13.13029	17	-	-	-	-

Statistical Comparison of Results Obtained from the Three Developed Methods

A, B and C methods were compared using one-way analysis of variance (ANOVA), and as a conclusion, no significant difference was found between the methods, as the F_{cal} value was found less than F_{tab} and the results of one-way ANOVA are given in Tables 4 and 5.

CONCLUSION

Three spectrophotometric methods (first derivative spectroscopic, dual-wavelength, and Q-absorbance ratio) were developed to simultaneously estimate ALC and KTC in their pharmaceutical dosage formulation. The methods were precise and accurate as can be reflected from validation data and were successfully applied to estimate ALC and KTC in the formulation. The one-way ANOVA results show no significant difference between assay results obtained from these three methods. Hence the proposed methods can be used in routine analysis of ALC and KTC with relatively less expensive and simple instrumentation.

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