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Research Article

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Extractive Spectrophotometric Determination of Azelastine Hydrochloride in Pure Form and Pharmaceutical Formulations

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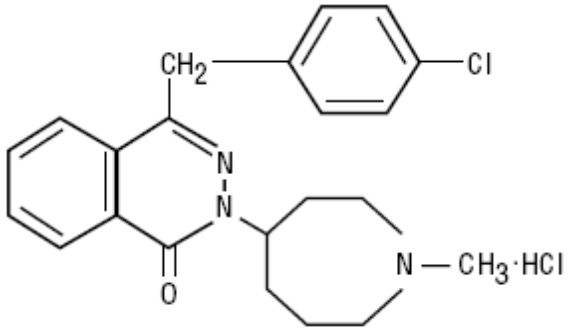
Abstract: Three simple, rapid, sensitive and extractive spectrophotometric methods were developed for the determination of azelastine hydrochloride (AZL) in pure form and pharmaceutical formulations. The methods are based on the formation of ion-pair complex between AZL and bromocresol green (BCG), methyl orange (MO) and alizarin red S (ARS) in acidic buffer solutions. The formed complexes were extracted with chloroform and methylene chloride using (BCG or MO) and ARS, respectively and measured at 418, 427 and 425 nm for BCG, MO and ARS, respectively. The experimental analytical parameters have been carefully optimized to achieve the highest sensitivity. Beer's law was obeyed over the concentration ranges 1.0–20 μg mL^{-1} AZL with correlation coefficient of ≥ 0.9994. The molar absorptivity, Sandell sensitivity, limits of detection and limits of quantification were calculated. A Job's plot of the absorbance versus the molar ratio of AZL to each of dyes under consideration indicated (1 : 1) ratio and the conditional stability constant (K_f) of the complexes have been calculated. The proposed methods have been applied successfully for to the determination of AZL in pharmaceutical formulations with good accuracy and precision and without interference from common additives Statistical comparison of the results obtained by the proposed methods with the reference method showed excellent agreement and indicated no significant difference in accuracy and precision.

Keywords: Azelastine hydrochloride; Spectrophotometry; Ion pair complex; pharmaceutical formulations.

1. INTRODUCTION

Azelastine HCl is 4-(4-chlorobenzyl)-2-[(4RS)-1-methylhexahydro-1H-azepin-4-yl]phthalazin-1(2H)-one hydrochloride [1] (Scheme. 1). It is an intranasal antihistamine indicated for allergic rhinitis, conjunctivitis and vasomotor [2]. Few analytical methods were reported for its determination including electrochemical methods [3, 4, 5], thin layer chromatography (TLC) [6, 7], high performance liquid chromatography (HPLC) [8–12] and capillary electrophoresis [13, 14]. Moreover thermal analysis were reported for determination of AZL [15]. However, all these methods either involve costly equipment or

tedious experimentation and time consuming for the analysis of AZL. For routine analysis of AZL, a simple, rapid and cost effective analytical method was required. The spectrophotometric technique continues to be the most preferred method for the assay of different classes of drugs in pure, pharmaceutical formulations and biological samples, due to its simplicity and reasonable sensitivity with significant economic advantages. There are few spectrophotometric methods have been developed for the estimation of AZL in dosage forms [3, 16, 17]. These methods were associated with some major drawbacks such as decreased selectivity due to measurement in UV region and/or decreased simplicity of the assay procedure. For these reasons, it was worthwhile to develop a new simple and selective spectrophotometric method for the determination of AZL in its dosage forms.



Scheme 1. The chemical structure of azelastine hydrochloride (AZL)

In the present work, we report the development of accurate and precise extractive spectrophotometric methods based on the formation of ion–pair complexes between AZL and some dyes BCG, MO and ARS. The absorbance measurements were measured at optimum wavelengths. The proposed methods were applied successfully for the determination of AZL in pure and dosage forms. No interference was observed from the additives. The proposed methods have been demonstrated to be superior to the previously reported methods with respect to speed, simplicity, sensitivity, cost effectiveness and can be adopted by the pharmaceutical laboratories for industrial quality control. These methods were validated by the statistical data.

2. EXPERIMENTAL

2.1. Apparatus

All absorption spectra were made using Kontron Unikon 930 (UV-Visible) spectrophotometer (German) with a scanning speed of 200 nm min^{-1} and a band width of 2.0 nm, equipped with 10 mm matched quartz cells. The pH values of different buffer solutions were checked using a Hanna pH-meter instrument (pH 211) (Romania) equipped with a combined glass-calomel electrode.

2.2. Materials and Reagents

All reagents and chemicals used were of analytical or pharmaceutical grade and all solutions were prepared fresh daily.

2.2.1. Materials

Azelastine hydrochloride (AZL) (purity 99.0%) was kindly provided by European Egyptian Pharm Co., Egypt. Allergodil® nasal spray labeled to contain 1.0 mg azelastine-HCl per mL (MEDA Pharma GmbH & Co. KG, Germany) and Azelast eye drops, labeled to contain 0.5 mg azelastine-HCl per mL (El-Kahira Pharm and Chem Ind Co., EPCI, Egypt) were purchased from the local market.

Standard Drug Solutions

A stock standard solutions (100 µg mL^{-1}) and (1.0×10^{-3} M) of AZL were prepared by dissolving 10 mg of pure drug in 20 mL of 50% methanol in a 100 mL volumetric flask and sonicated for 15 minutes and completed to 100 mL with bidistilled water. The standard solutions were kept in an amber coloured bottle and stored in a refrigerator when not in use.

2.2.2. Reagents

Bromocresol green (BCG), methyl orange (MO) and alizarine red S (ARS) (BDH Chemicals LTD, Poole, England) and used without further purification. Stock solutions (1.0×10^{-3} M) of reagents were prepared by dissolving the appropriate weight of each reagent in10 mL of 96% ethanol and diluted to 100 mL with bidistilled water. These solutions were kept in the refrigerator.

Series of buffer solutions of KCl–HCl (pH=1.5-4.2), NaOAc–HCl (pH=2.0-4.9), NaOAc–AcOH (pH=3.0-5.6) and potassium hydrogen phthalate–HCl (pH=2.0-7.0) were prepared by following the standard methods [18]. The pH of each solution was adjusted to an appropriate value by addition of 0.2 M hydrochloric acid or sodium hydroxide with the help of pH meter. Freshly prepared solutions were always employed. Chloroform and methylene chloride (BDH), anhydrous sodium sulfate (Prolabo), methanol (BDH).

2.3. General Procedure

Aliquots of (0.1–2.0 mL) the standard drug solution (100 µg mL^{-1}) were transferred to 10 mL measuring flasks and added 2.0 mL acetate buffers of pH 2.5, 3.5 and 4.0 using BCG, MO and ARS, respectively then add 2.0 mL of each reagent solutions (1.0×10^{-3} M). The mixture was extracted with 10 mL chloroform and methylene chloride using (BCG or MO) and ARS, respectively by shaking for 2.0 min, then allowed to stand for clear separation of the two phases and the organic layer was passed through anhydrous sodium sulphate. The absorbance of the yellow colored complexes were measured at 418, 427 and 425 nm, using BCG, MO and ARS, respectively against corresponding reagent blank similarly prepared. All measurements were made at room temperature (25 ±2°C). The procedures were repeated for other analyte aliquots and calibration plots were drawn to calculate the amount of drug in unknown analyte samples.

2.4. Assay Procedure for Pharmaceutical Formulations

An aliquot volume of syrup or drops equivalent to 5.0 mg of AZL were pipetted into 50-mL volumetric flask and completed to volume with methanol (50% v/v). Aliquots covering the working concentration range of drug were transferred into 10 mL volumetric flasks. Proceed as described under "General Procedure" adopting any of the three methods. "The nominal content of the syrup or drops was calculated using the corresponding regression equations of the calibration graphs."

2.5. Stoichiometric Relationship

The stoichiometric ratios of the ion-pairs formed between AZL and the reagents were determined by applying the continuous variation [19] and the molar ratio [20] methods at the optimum wavelengths. In continuous variation method, equimolar solutions were employed: a 5.0 x 10^{-4} M standard solution of drug and 5.0 x 10^{-4} M solution of dye were used. A series of solutions was prepared in which the total volume of the studied drugs and the dye was kept at 2.0 mL. The drug and reagent were mixed in various complementary proportions (0.2:1.8, 0.4:1.6, 0.6:1.4, 0.8:1.2, 1.0:1.0, 1.2:0.8, 1.4:0.6, 1.6:0.4, 1.8:0.2) and completed to volume in a 10 mL calibrated flask with the appropriate solvent for extraction following the above mentioned procedure. In the molar ratio method, the concentration of AZL was kept constant 1.0 mL of (5.0 x10 $^{-4}$ M) while that of dyes (5.0 x10 $^{-4}$ M) is regularly varied (0.2 – 2.4 mL). The absorbance of the prepared solutions measured at optimum condition and at optimum wavelength for each complex.

3. RESULTS AND DISCUSSION

3.1. Absorption Spectra

The nitrogenous drugs are present in positively charged protonated forms and anionic dyes of sulphonpthalein group present mainly in anionic form at pH ≥ 2.5. So when treated with an acid dye at pH range (2.8-5.0) of acidic buffer solutions, a yellow ion-pair complex which is extracted with organic solvent is formed. The absorption spectra of the ion-pair complexes, which were formed between AZL and reagents were measured in the range 350–550 nm against the blank solution. The ion-pair complexes of AZL and BCG, MO and ARS show maximum absorbance's at 418, 427 and 425 nm, respectively (Fig. 1).

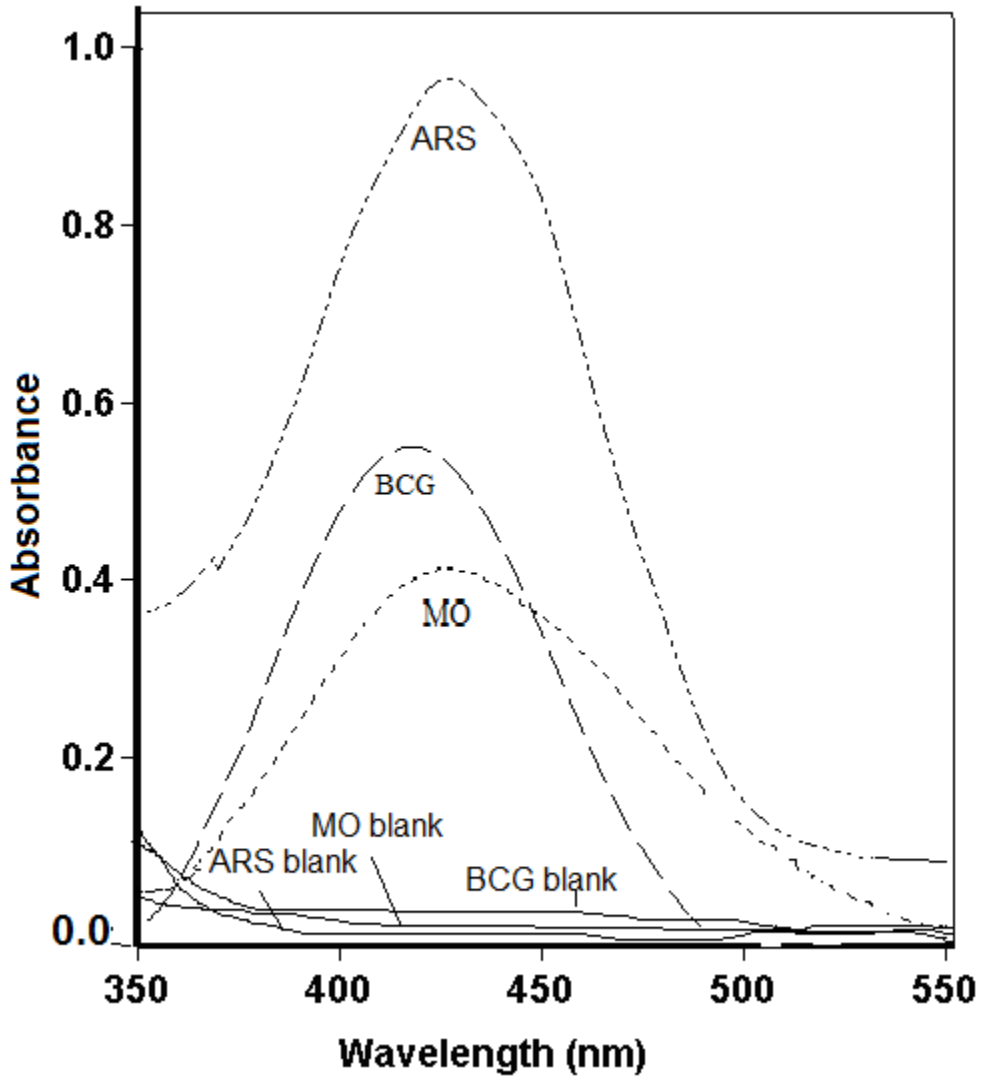


Figure 1. Absorption spectra of ion-pair complexes of (15 µg mL^{-1}) AZL with (1.0 x 10^{-3} M) reagents against reagent blank.

3.2. Optimum Reaction Conditions For Complex Formation

The optimization of the methods was carefully studied to achieve complete reaction formation, highest sensitivity and maximum absorbance.

3.2.1. Effects of pH on Ion-Pair Formation

The effect of pH on the drug–reagent complex was studied by extracting the colored complexes in the presence of various buffers. It was noticed that the maximum color intensity and highest absorbance value were observed in NaOAc- AcOH buffer of pH 2.5, 3.5 and 4.0 using BCG, MO and ARS, respectively (Fig. 2). In addition to the stability of the color without affecting the absorbance at the optimum pH values. Further, 2.0 mL of the buffers solutions gave maximum absorbance's and reproducible results.

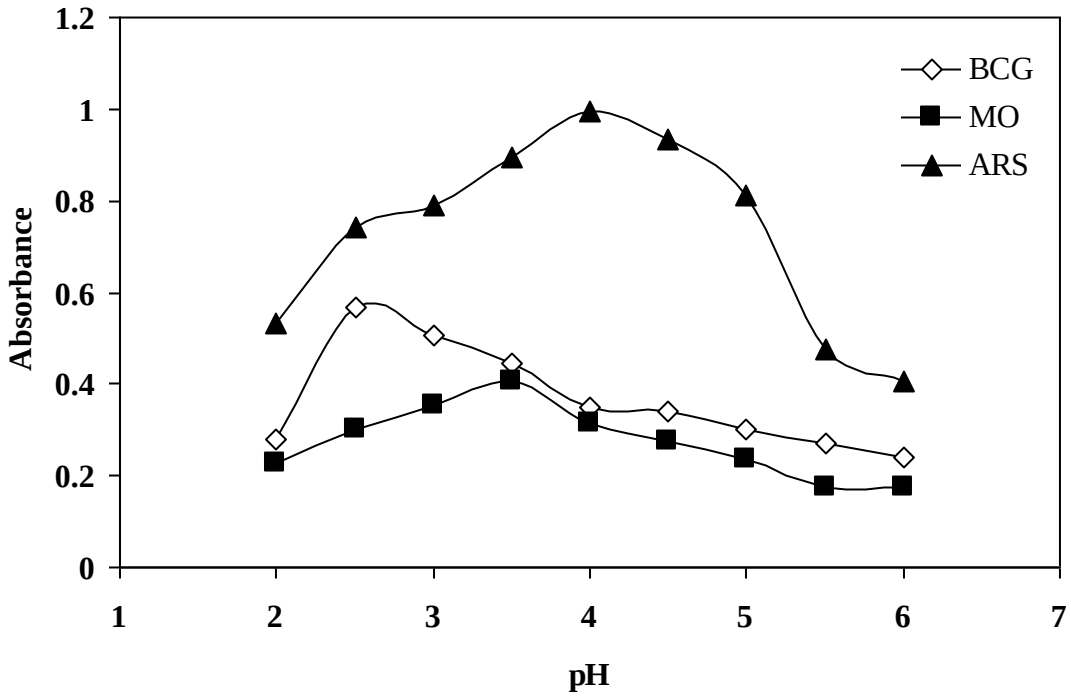


Figure 2. Effect of pH of buffer solution on ion pair complex formation between the AZL and (1.0 x 10^{-3} M) reagents.

3.2.2. Effect of Extracting Solvents

The effect of several organic solvents *viz*., chloroform, carbon tetrachloride, methanol, ethanol, acetonitrile, *n*-butanol, benzene, acetone, ethyl acetate, diethylether, toluene, dichloromethane and chlorobenzene were studied for effective extraction of the colored species from aqueous phase. Chloroform and methylene chloride were found to be the most suitable solvent for extraction of colored ion pair complexes for (BCG or MO) and ARS, respectively. Experimental results indicated that double extraction with total volume 10 mL slovent, yielding maximum absorbance intensity, stable absorbance for the studied drugs and considerably lower extraction ability for the reagent blank and the shortest time to reach the equilibrium between both phases.

3.2.3. Effects of Reagents Concentration

The effect of the reagents was studied by measuring the absorbance of solution containing a fixed concentration of AZL and varied amounts of the respective reagents. Maximum color intensity of the complex was achieved with 2.0 mL of 1.0×10^{-3} M reagents solutions. Although a larger volume of the reagent had no pronounced effect on the absorbance's of the formed ion-pair complex (Fig. 3).

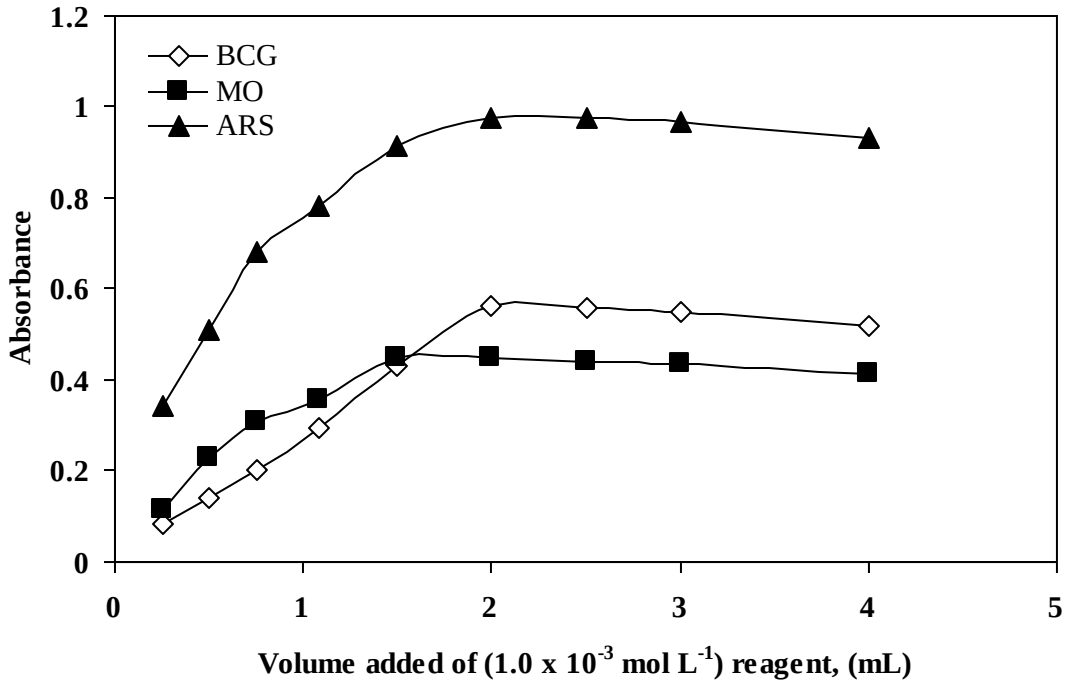


Figure 3. Effect of volume of (1.0 ×10^{-3} mol L^{-1}) reagent on the ion pair complex formation with AZL.

3.2.4. Effect of Time and Temperature

The optimum reaction time was investigated from 0.5 to 5.0 min by following the color development at ambient temperature (25 ± 2°C). Complete color intensity was attained after 2.0 min of mixing for all complexes. The effect of temperature on colored complexes was investigated by measuring the absorbance values at different temperatures. It was found that the colored complexes were stable up to 30°C. At higher temperatures, the drug concentration was found to increase due to volatile nature of the solvent. The absorbance remains stable for at least 12 h at room temperature all drug-dye reagents.

3.3. Stoichiometric Relationship

The stoichiometric ratio between AZL and dye in the ion-pair complexes was determined by the continuous variations method (Fig. 4). Job's method of continuous variation of equimolar solutions was employed: a 5.0 × 10^{-4} M standard solution of drug base and 5.0 × 10^{-4} M solution of BCG, MO or ARS were used. A series solution was prepared in which the total volume of drug and reagent was kept at 2.0 mL in the total volume of 10 mL of aqueous layer. The absorbance of extracted ion-pair in each instance was measured at the optimum wavelength and plotted against the mole fraction of the drug. The results indicate that the molar ratio of (drug : dye) is 1:1 ion-pairs are formed through the electrostatic attraction between positive protonated AZL^{+} and negative BCG^{-}, MO^{-} and ARS^{-}. The extraction equilibrium can be represented as follows:

$$AZL^{+}_{(aq)} + D^{-}_{(aq)} \longleftrightarrow AZL^{+}D^{-}_{(aq)} \longleftrightarrow AZL^{+}D^{-}_{(org)}$$

Where AZL^{+} and D^{-} represent the protonated AZL and the anion of the dye, respectively, and the subcript (aq) and (org) refer to the aqueous and organic phases, respectively (Scheme 2).

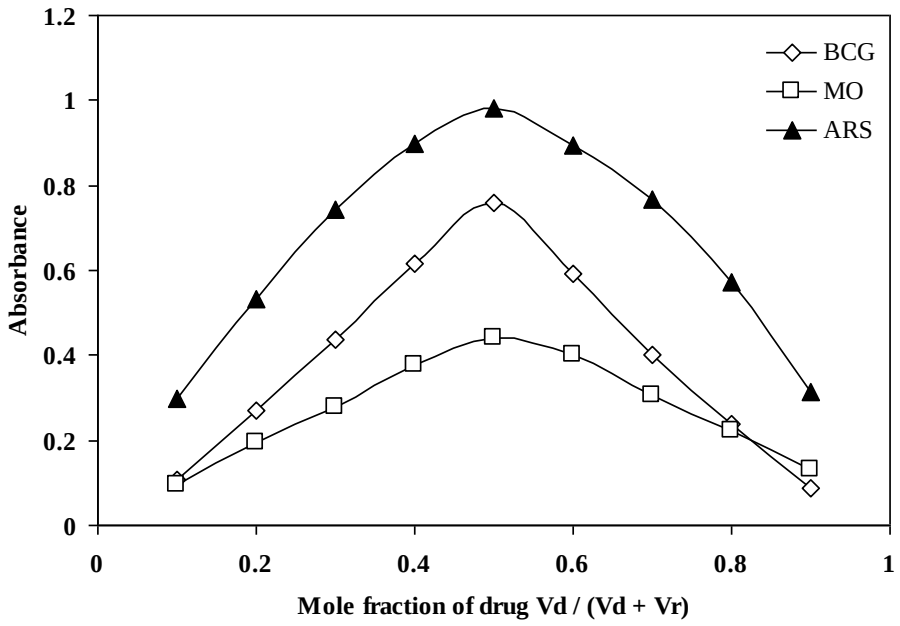
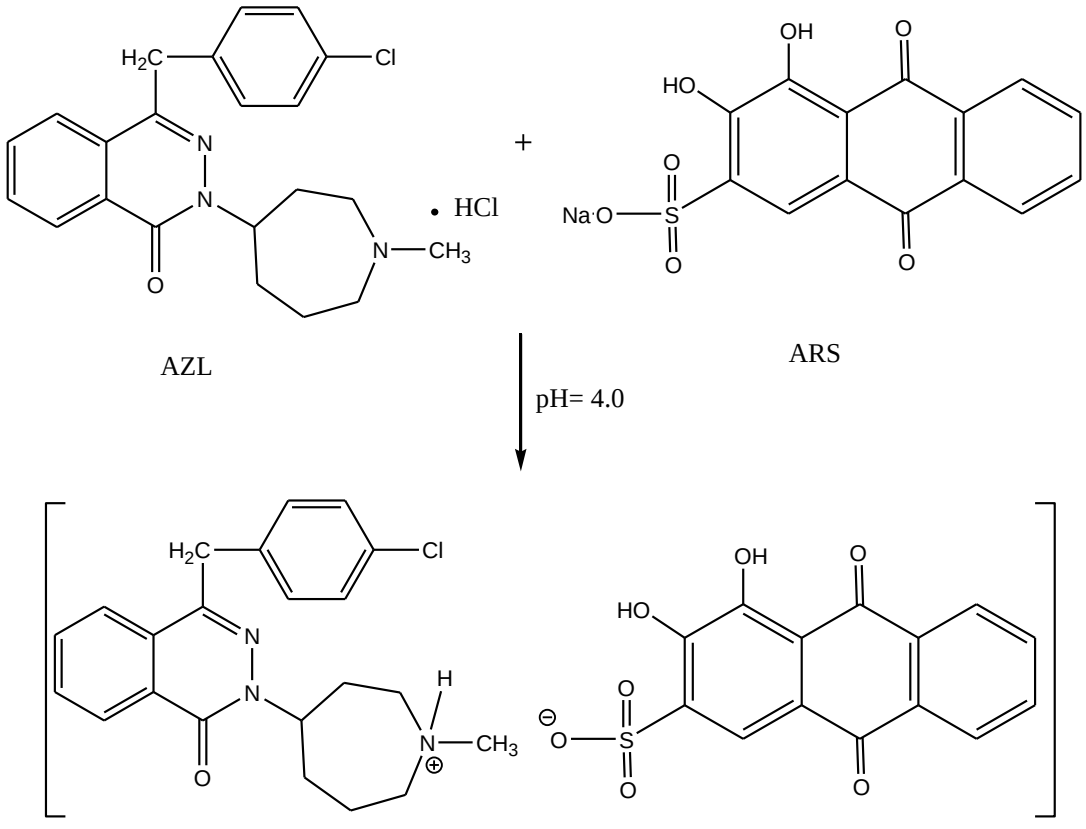


Figure 4. Job's method of continuous variation graph for the reaction of AZL with the studied dyes, [drug] = [dye] = (5.0×10^{-4} M).



1:1 yellow ion-pair complex of AZL-ARS measured at 425 nm

Scheme 2. Proposed reaction mechanism for the ion pair complex formation between AZL and ARS.

Table 1. Statistical analysis of calibration graphs and analytical data in the determination of AZL using the proposed methods.

Parameters	BCG	MO	ARS
Wavelengths λ_{max} (nm)	418	427	425
pH	2.5	3.5	4.0
Beer's law limits (µg mL^{-1})	1.0-20	1.0-16	2.0-20
Molar absorptivity ε, (L mol^{-1} cm^{-1}) x 10^4	1.1971	1.113	2.4428
Sandell's sensitivity (ng cm^{-2})	34.95	37.05	17.13
log K_f	5.415 ± 0.19	4.978 ± 0.18	4.912 ± 0.20
Regression equation [a]			
Intercept (*a*)	- 0.0007	0.0024	- 0.0117
Slope (b)	0.0278	0.026	0.061
Correlation coefficient (*r*)	0.9994	0.9995	0.9995
LOD (µg mL^{-1}) [b]	0.23	0.29	0.56
LOQ (µg mL^{-1}) [b]	0.77	0.97	1.87
Mean ± SD	99.90 ± 0.94	100.24 ± 1.28	100.30 ± 1.26
RSD%	0.94	1.28	1.26
RE%	0.99	1.35	1.33
t-test [c]	0.90	0.34	0.26
F- test [c]	1.50	1.24	1.20

[a] *$A = a + bC$, where C is the concentration in µg mL^{-1}, A is the absorbance units.*
[b] *LOD, limit of detection; LOQ, limit of quantification; ε ,molar absorptivity.*
[c] *The theoretical values of t and F at P= 0.05 are 2.571 and 5.05, respectively*

3.4. Conditional Stability Constants (K_f) of Ion-Pair Complexes

The stability of the ion-pair complexes was evaluated. The formation of the ion-pairs were rapid and the yellow color extracts were stable at least for 12 h for drug-dye without any change in color intensity and the maximum absorbance at room temperature. The conditional stability constants (K_f) of the ion-pair complexes for the studied drug were calculated from the continuous variation data using the following equation [21]:

$$K_f = \frac{A/A_m}{[1 - A/A_m]^{n+1} C_M (n)^n}$$

where A is the observed maximum absorbance, and A_m is the absorbance value corresponding to intersection of the two tangents of the curve, C_M is the mole concentration corresponding to maximum absorbance and n is the stoichiometry with which dye ion associates with drugs. The log K_f values for

drug-dye ion-pair associates were calculated in Table 1.

Table 2. Intra-day and Inter-day precision and accuracy data for AZL obtained by the proposed methods.

Method	Added (μgmL^{-1})	Intra-day				Inter-day			
		Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence limit [b]	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence limit [b]
BCG	5.0	99.40	0.37	-0.60	4.97 ± 0.019	98.90	0.31	-1.10	4.945 ± 0.016
	10	99.20	0.62	-0.80	9.92 ± 0.069	99.40	0.59	-0.60	9.94 ± 0.62
	20	100.10	1.06	0.10	20.02 ± 0.233	99.70	1.10	-0.30	19.94 ± 0.230
MO	4.0	99.10	0.47	-0.90	3.964 ± 0.02	99.00	0.36	-1.0	3.96 ± 0.015
	8.0	99.40	0.62	-0.60	7.952 ± 0.052	98.70	0.73	-1.30	7.896 ± 0.061
	16	99.70	0.86	-0.30	15.952 ± 0.144	99.50	0.91	-0.50	15.92 ± 0.152
ARS	5.0	99.80	0.40	-0.20	4.99 ± 0.021	99.40	0.41	-0.60	4.97 ± 0.021
	10	99.50	0.59	-0.50	9.95 ± 0.062	99.80	0.89	-0.20	9.98 ± 0.093
	20	100.50	1.16	0.10	20.10 ± 0.245	100.40	1.10	0.40	20.08 ± 0.232

[a] *Mean of six determination, RSD%, percentage relative standard deviation; RE%, percentage relative error.*
[b] *Confidence limit at 95% confidence level and five degrees of freedom (t = 2.571).*

3.5. Method of Validation

3.5.1. Linearity

At described experimental conditions for AZL determination, standard calibration curves with reagents were constructed by plotting absorbance vs. concentration of AZL. The statistical parameters were given in the regression equations calculated from the calibration graphs A = a C + b, where A is the absorbance and C is concentration in $\mu g\ mL^{-1}$. The linearity of calibration graphs was proved by the high values of the correlation coefficient (*r*) and the small values of the *y*-intercepts of the regression equations. The apparent molar absorptivity of the resulting colored ion-pair complexes and relative standard deviation of response factors for each proposed spectrophotometric method were also calculated and recorded in Table 1. The molar absorptivity of ARS > BCG > MO ion-pair complexes.

3.5.2. Sensitivity

The limits of detection (LOD) and quantitation (LOQ) for the proposed methods were calculated using the following equation [21, 22]:

$$LOD = 3s / k \text{ and } LOQ = 10\ s / k$$

where *s* the standard deviation of the response of the blank or the standard deviation of intercepts of regression lines and *k* is the sensitivity, namely the slope of the calibration graph. In accordance with the

formula, the limit of detection were found to be 0.23, 0.29 and 0.56 µg mL^{-1} for BCG MO and ARS methods, respectively.
According to this equation, the limit of quantitation were found to be 0.77, 0.97 and 1.87 µg mL^{-1} for BCG MO and ARS methods, respectively.

3.5.3. Accuracy and Precision

Specificity of ion-pair reaction and selective determination of AZL which was the basic nitrogenous compound with acid dyes could be possible. Percentage relative standard deviation (RSD %) as precision and percentage relative error (RE %) as accuracy of the suggested methods were calculated. Precision was carried out by six determinations at four different concentrations in these spectrophotometric methods. The percentage relative error calculated using the following equation:

$$RE\ \% = [(founded - added) / added] \times 100$$

The inter-day and intra-day precision and accuracy results are shown in (Table 2). These results of accuracy and precision show that the proposed methods have good repeatability and reproducibility.

3.5.4. Robustness and Ruggedness

For the evaluation of the method robustness, some parameters were interchanged; pH, dye concentration, wavelength range, and shaking time. The capacity remains unaffected by small deliberate variations. Method ruggedness was expressed as RSD% of the same procedure applied by two analysts and in two different instruments on different days. The results showed no statistical differences between different analysts and instruments suggesting that the developed methods were robust and rugged (Table 3).

Table 3. Method robustness and ruggedness expressed as intermediate precision (RSD %) for AZL-BCG ion pair complex.

AZL taken (µg mL^{-1})	Robustness			Ruggedness	
	Parameters altered			Inter-analysts (RSD / %) (N=3)	Inter-instruments (RSD / %) (N=3)
	Volume of BCG[a]	Volume of buffer [b]	Reaction time [c]		
5.0	1.71	1.30	1.80	1.50	1.10
10	1.10	1.40	1.70	1.40	1.80
20	1.20	1.90	1.30	2.10	1.70

[a] *The volumes of BCG dye used were 2.0 ± 0.2 mL.*
[b] *The volumes of buffer used were 2.0 ± 0.2 mL.*
[c] *The reaction times were 2.0 ± 0.5 min.*

3.5.4. Effects of Interference

To assess the usefulness of the method, the effect of diluents, excipients and additives which often accompany AZL in its formulations (nasal spray and drops) was studied. The results indicated that there is no interference from excipients and additives, indicating a high selectivity for determining AZL in its formulations.

Table 4. Application of the standard addition technique for the determination of AZL in dosage forms using the proposed methods.

Sample	Taken (μgmL^{-1})	BCG		MO		ARS		Ref. method [23]
		Added (μgmL^{-1})	Recovery [a] (%)	Added (μgmL^{-1})	Recovery [a] (%)	Added (μgmL^{-1})	Recovery [a] (%)	
Allergodil® nasal spray	2.0	-	99.00	-	99.20	-	99.80	
		2.0	99.50	2.0	99.70	2.0	99.40	
		6.0	100.30	4.0	99.00	6.0	99.90	
		10	99.10	8.0	99.50	10	98.80	
		14	98.80	12	99.30	14	98.60	
		18	99.60	14	100.50	18	99.90	
Mean ± SD			99.38 ± 0.54		99.53± 0.532		99.40 ± 0.576	99.52 ± 1.02
R.S.D%			0.54		0.532		0.576	1.02
V			0.294		0.283		0.332	1.03
S.E			0.221		0.217		0.222	0.42
t-value [b]			0.27		0.019		0.2290	
F-value [b]			3.57		3.68		3.14	
Azelast eye drops	2.0	-	99.00	-	99.20	-	99.40	
		2.0	99.70	2.0	99.80	2.0	99.80	
		6.0	99.20	4.0	98.50	6.0	99.10	
		10	98.40	8.0	99.40	10	98.20	
		14	100.60	12	99.10	14	98.70	
		18	99.40	14	100.90	18	101.10	
Mean ± SD			99.38 ± 0.739		99.48± 0.813		99.38 ± 1.007	99.26 ± 1.11
R.S.D%			0.739		0.813		1.01	1.10
V			0.546		0.662		1.014	1.23
S.E			0.302		0.332		0.411	0.45
t-value [b]			0.201		0.357		0.1790	
F-value [b]			2.26		1.86		1.22	

[a] *Average of six determinations.*

[b] *The theoretical values of t and F are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p= 0.05).*

3.6. Applications to Pharmaceutical Formulations

The proposed methods have been successfully applied to the determination of AZL in dosage forms (Allergodil® nasal spray and Azelast eye drops). The results in Table 4 showed that the excipients in the dosage forms do not interfere. A statistical comparison of the results for determination of AZL from the same batch of material by the proposed and reference method [23] is shown in Table 4. The results agreed well with the label claim and also are in agreement with the results obtained by the reference method. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed and reference method at the 95 % confidence

level with respect to accuracy and precision (Table 4).

To ascertain the accuracy and validity of the proposed methods, recovery experiment was performed *via* standard addition technique. To a fixed and known amount of AZL in dosage form (pre-analysed), pure drug was added at different concentrations and the total was found by the proposed methods. Results of this study presented in Table 4 indicated that the commonly added excipients did not interfere in the assay.

4. CONCLUSION

This paper describes the application of extractive ion–pair complexation reaction with acid dyes for the quantification of AZL in pure form and pharmaceutical formulations. Compared with the existing visible spectrophotometric methods, the proposed methods have the advantages of relatively simple, rapid, cost-effective, free from auxiliary reagents and more sensitive for determination AZL in pure form and pharmaceutical formulations. Moreover, the proposed methods are free from tedious experimental steps such as heating unlike the previously reported spectrophotometric methods cited earlier. The most attractive feature of these methods is its relative freedom from interference by the usual diluents and excipients in amounts far in excess of their normal occurrence in pharmaceutical formulations. The statistical parameters and the recovery data reveal high precision and accuracy of the methods besides being robust and rugged. Therefore, the validated method could be useful for routine quality control assay of the studied drug in pure form and pharmaceutical formulations.

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