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Determination of Albendazole in the Presence of its Alkaline Degradation Product Using TLC-Densitometric and Chemometric Methods: A Comparative Study

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ABSTRACT
Four simple, specific, accurate and precise methods have been used for the determination of albendazole (ALB) in the presence of its alkaline degradation product. Method A, a densitometric one, after separation on silica gel plate using dichloromethane: methanol (90:10 v/v) as a mobile phase and the spots were scanned at 232 nm. Beer's law was obeyed over the concentration range of 1–5 µg/spot. Methods B, C and D, chemometric-assisted methods, including classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS), albendazole was analyzed with mean accuracy of 99.94±0.159, 99.93±0.159 and 99.93±0.158 for CLS, PCR and PLS, respectively. A comparative study between the methods was done showing the advantages and the disadvantages of each method. These methods are suitable as stability indicating methods for the simultaneous determination of albendazole in the presence of its alkaline degradation product either in bulk powder or in pharmaceutical formulations.
Keywords: albendazole, TLC-densitometry, CLS, PCR, PLS

INTRODUCTION

ALB, is a broad spectrum anthelmintic. It is used for the treatment of Threadworm, Hookworm and Tape-worm [1-3]. ALB, **Figure 1,** chemically known as methyl [5-(propylthio)-1H-benzimidazol-2-yl] carbamate [3-5], is widely used as an anthelmintic having a wide spectrum of activity. The drug is official in British Pharmacopoeia [4], which describes a potentiometric titration with perchloric acid in formic acid–acetic acid medium. The development of reliable and affordable procedures for assay of drug substances either as pure drug or in combination remains a major research area in today's pharmaceutical care and practice [6]. To the best of our knowledge, the estimation of the drug in pure form using non-

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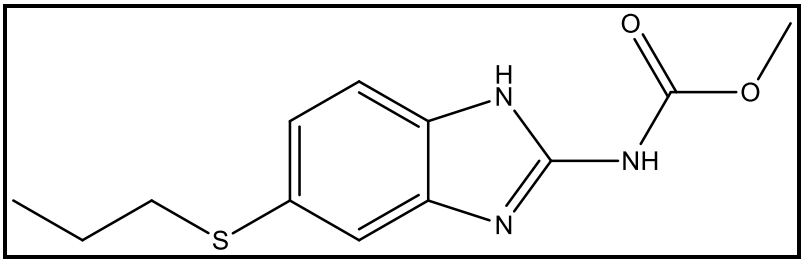

Figure 1. Structural formula of albendazole

aqueous titration is described in British Pharmacopoeia. Chemical structure of ALB is given in **Figure 1**. ALB is effective in the treatment of echinococcosis, hydrated cysts and neurocysticercosis. Several techniques such as HPLC, HPLC with fluorescence detention, LC-MS, capillary electrophoresis, spectrophotometric, titrimetric and flow injection analysis for the estimation of ALB alone and with its major metabolites had been reported [7]. These methods used for the estimation are bit time consuming, tedious and expensive. The developed methods were validated as per ICH guidelines and USP requirements [8]. Suitable statistical tests were performed on validation data [9,10]. Visible spectrophotometric procedures were reported [11-15]. Thin-layer chromatography (TLC) is one of the most versatile and widely used separation methods in chromatography. Speed of separation (development time), high sensitivity and good reproducibility, all result from the higher quality of chromatographic layers and the continual improvement in instrumentation. In addition, TLC has remained relatively inexpensive and one can easily see why it is still popular today. The main advantage of this technique is its ability to analyze several samples simultaneously using a small quantity of mobile phase, thus reduces time and cost of analysis. Moreover, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents thereby, reducing possibility of environment pollution [16]. As a result, it has become so well established that it is sometimes the technique of choice for the assay of drugs in binary or in multi-component mixtures [17-23].

The CLS, PCR and PLS techniques are useful in spectral analysis because the simultaneous inclusion of many spectral wavelengths instead of the single wavelength used in derivative spectrophotometry has greatly improved the precision and predictive abilities of these multivariate calibrations.

The aim of the work was to develop simple, sensitive, accurate and precise assay methods for the estimation of ALB in tablets (either alone or in the presence of its degradation product).

EXPERIMENTAL

Apparatus

Shimadzu UV-Vis. 1650 Spectrophotometer, (Tokyo, Japan), equipped with 10 mm matched quartz cells was used. All measurements were done at medium sensitivity.

- Hot plate, Torrey pines scientific, USA.
- pH meter 3510 (Jenway,U.S.A.).
- Camag TLC scanner 3, with WINCATS computer software (Switzerland).
- Precoated TLC plates, silica gel 60 GF254 (20 × 20 cm), (Fluka chemie, Switzerland).
- Hamilton 50-µL microsyringe (Germany).
- UV lamp with short wavelength (254 nm) (Desega-Germany).
- Chromatographic tank (25 × 25 × 9 cm).
- Rotary evaporator (Scilogex-RE 100-pro, USA).
- FT-IR, Nicolet IR 200 (Thermo electron corporation, USA).
- NMR, Gemini-300 BB (Agilent, USA).
- GCMS-QP-1000 EX mass spectrometer at 70 ev (Shimadzu, Tokyo, Japan.

Materials and reagents

Pure sample

Albendazole: was kindly supplied by Eipico Company, Egypt, B.No. (RN0030612).

Pharmaceutical preparation

Alzental ® tablets: product of Eipico company, Egypt, B.No. (40708), labeled to contain 200 mg of albendazole per tablet purchased from local pharmacies.

Reagents and solvents

All reagents used were of analytical grade, solvents were of HPLC grade, water used throughout the procedure was freshly distilled.

- Methanol, HPLC grade (Sigma-Aldrich, Germany).
- Sodium hydroxide, analytical grade (El Nasr Pharmaceutical, Chemicals Company, Egypt).
- Hydrochloric acid, analytical grade (El Nasr Pharmaceutical, Chemicals Company, Egypt).
- Dichloro methane, (E. Merck, D-6100 Darmstadt, F.R. Germany).

Software

- UV-Probe personal spectroscopy software version 2.1. (SHIMADZU).
- All chemometric methods were implemented in Matlab R2013b (8.2.0.701).
- PLS, PCR, CLS, were carried out by using PLS toolbox software version 2.1.

Standard solutions

Standard stock solution of intact sample

A stock solution of albendazole (1 mg/ml) was prepared by dissolving 100 mg of albendazole in least amount of 0.1 N HCL and complete to100 ml with methanol.

Standard solution of degraded sample

Base-induced forced degradation was performed by adding 100 mg of albendazole to 100 mL 5 N NaOH and the solution was heated under reflux for 3 hours. The solution was then left to reach ambient temperature, neutralized to pH 7 by addition of 5 N HCl, evaporated to dryness under vacuum. The obtained residue was extracted with methanol containing 0.1 N HCL, filtered into 100 ml volumetric flask then the volume was adjusted by the same solvent. The obtained solution was claimed to contain (1000 µg/ml).

PROCEDURES

Chromatographic conditions

Analysis was performed on precoated 20 × 20 cm TLC aluminum silica gel 60 GF_{254} plates. Samples were applied to the plates using Hamilton microsyringe (50- µL). Plates were spotted 1 cm apart from each other and 1 cm apart from the bottom edge. The chromatographic tank was pre-saturated with the mobile phase for 20 min, then developed by ascending chromatography using dichloromethane:methanol (90:10, v/v) as a mobile phase. The plates were air dried, detected under UV- lamp and scanned under the following conditions:

- Silt dimensions: 6.0 × 0.3 µm.
- Wavelength: 232 nm.
- Scanning speed: 20 mm/s.
- Data resolution: 100 nm / step.
- Measurement mode: absorption.
- Result output: chromatogram and integrated peak area.

General procedure: (for TLC-densitometric method)

In a series of 10 ml volumetric flasks, aliquots of standard albendazole solution (1 mg/ml) equivalent to (1-5 mg) drug were transferred and diluted to volume with methanol. 10 µL of each solution were applied to a TLC plate following the above mentioned specific chromatographic conditions and scanned at 232 nm. Calibration graph was constructed by plotting area under the peak versus the corresponding drug concentrations in µg/spot and the regression equation was computed.

Specificity: (for TLC-densitometric method)

The general procedure of the method was repeated using aliquots of intact albendazole standard solution (1 mg/ml) containing (1-5 mg) with aliquots of albendazole degradate standard solution (1 mg/ml) containing (5-1 µg) and then diluted to the volume with methanol. The intact drug concentrations were calculated from the corresponding regression equation.

General procedure: (for chemometric methods)

Experimental design

Brereton [24] constructed multilevel multifactor experimental design was applied for the construction of the calibration and validation sets. A five-levels, two factors experimental design was used in which 0.8, 0.9, 1, 1.1 or 1.2 mL aliquots of both intact and degraded form of ALB working solutions were combined and diluted to 10 mL with water. The concentrations details are given in **Table 1**. The absorption spectra of the prepared mixtures were recorded over the wavelength range 200-400 nm with 1 nm interval thus the produced spectral data matrix has 25 rows representing different samples and 201 columns representing wavelengths (25 x 201). For construction of the models, to build the CLS, PCR and PLS models, feed the computer with the absorbance and concentration matrices for the training set, use the training set absorbance and concentration matrices using Matlab® version R2013b (8.2.0.701), together with PLS-Toolbox 2.1. software for the calculations. The concentrations were calculated from the corresponding regression equations.

Analysis of pharmaceutical preparation

For TLC-densitometric method: Ten Alzental ® tablets were accurately weighed and finely powdered. Appropriate weight of powder equivalent to 100 mg of albendazole was accurately weighted, transferred to 100-ml volumetric flask and the volume was made up to 75 ml with methanol. The solution was shaken vigorously for 15 min then sonicated for 30 min and then filtered. The volume was completed to 100 ml with methanol to obtain a concentration of 1mg/ml. Repeat the general procedure using aliquots covering the working concentration range. Determine the drug concentrations from the corresponding regression equation.

For chemometric methods: The same under TLC-densitometric method till preparation of stock solution, then proceed as described under "general procedure for chemometric methods".

RESULTS AND DISCUSSION

In the present study, simple and sensitive stability indicating TLC-densitometric and chemometric procedures were suggested for the selective quantitative determination of ALB in presence of its alkaline degradate.

Table 1. Concentrations of Intact and Degraded ALB mixtures used in chemometric methods

No. of Mix	Intact (µg/ml)	Degradate (µg/ml)
1	15	15
2	15	5
3	5	5
4	5	25
5	25	10
6	10	25
7	25	15
8	15	10
9	10	10
10	10	20
11	20	25
12	25	20
13	20	15
14	15	25
15	25	25
16	25	5
17	5	20
18	20	5
19	5	15
20	15	20
21	20	20
22	20	10
23	10	5
24	5	10
25	10	15

Degradation of ALB [25]

Accelerated degradation of ALB was achieved upon heating under reflux with 5 M sodium hydroxide for 3 hours. For isolation of ALB degradation product from the reaction medium, the solution after refluxed for 3 hours, cooled, neutralized, evaporated under vacuum till dryness and extracted with methanol. The obtained solution was tested by TLC on silica gel 60 GF254 plates. Separation of the intact drug and its corresponding degradation product was achieved by using mobile phase consisting of dichloromethane:methanol (90:10 v/v) and UV detection at 232 nm.

Confirmation of degradation product

IR spectrum of the intact albendazole showed highly intense band at 3327 cm^{-1} for NH of amide group , 2949 cm^{-1} of -CH aliphatic and 1631 cm^{-1} of C=O of amide group as shown in **Figure 2**. While the IR of the degradate showed highly intense band at 3467 of NH_2 group as shown in **Figure 3.**

The H^1NMR of the intact albendazole showed characteristic protons of the O-CH_3 group at 3.76 ppm, protons of the NH of amide group and NH of albendazole nucleus at 11.69 ppm, as shown in **Figure 4**. While the H^1NMR of the degradate showed a characteristic protons of NH_2 group at 6.22ppm, protons of NH of albendazole nucleus at 10.85 ppm and disappearance of protons of the O-CH_3 group, and as shown in **Figure 5**.

Mass interpretation for degradate reveals that molecular weight of ALB degradate is 207.15 as shown in **Figure 6.** The predicted structural formula of ALB degradate is shown in **Figure 7.**

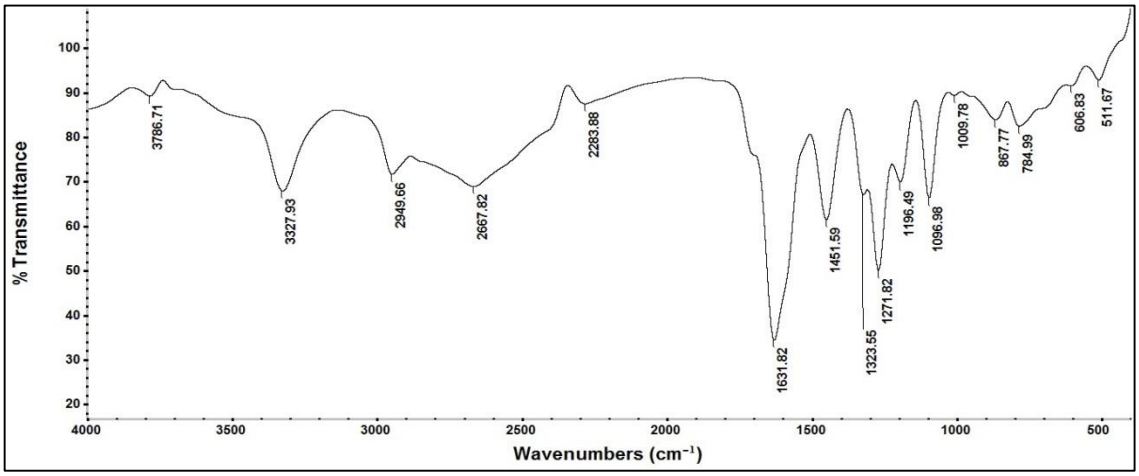

Figure 2. IR spectrum of ALB on KBr disc

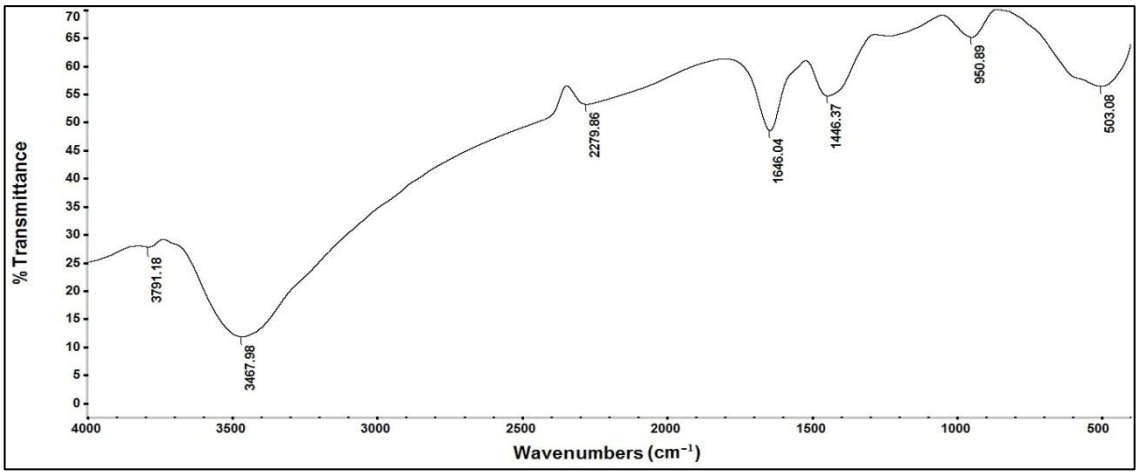

Figure 3. IR spectrum of ALB degradate on KBr disc

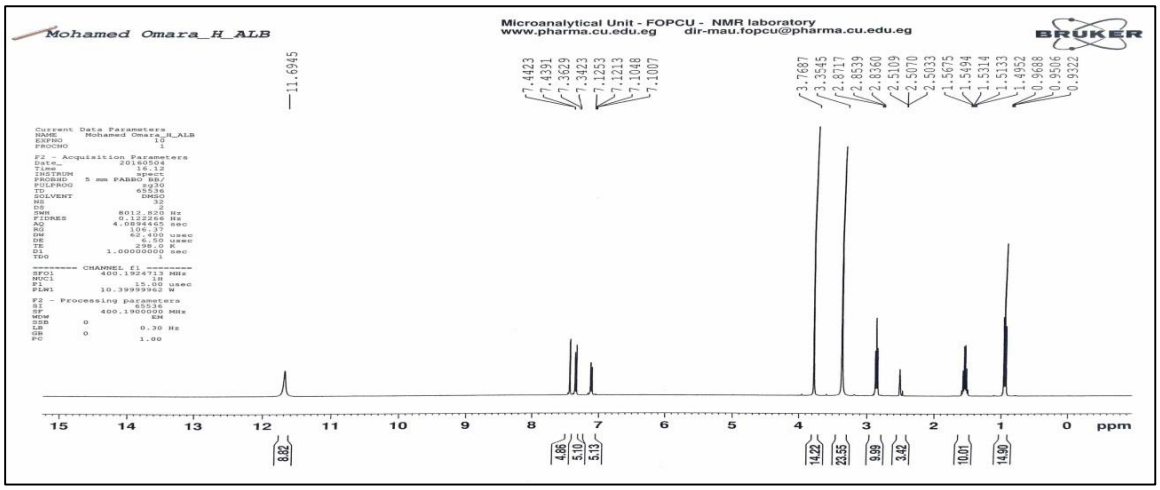

Figure 4. H^1NMR spectrum of ALB in DMSO

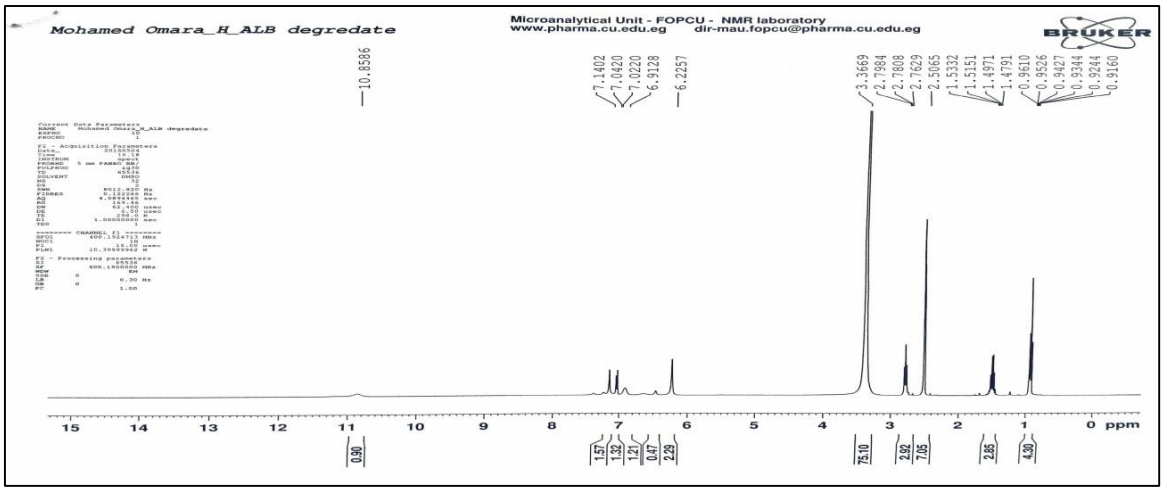

Figure 5. H^1NMR spectrum of ALB degradate in DMSO

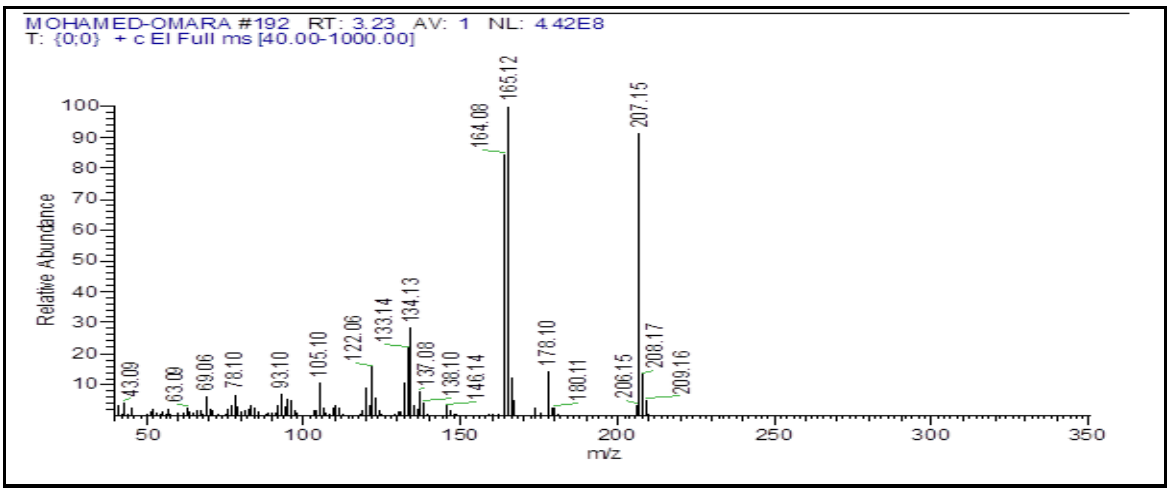

Figure 6. Mass spectrum of ALB degradate

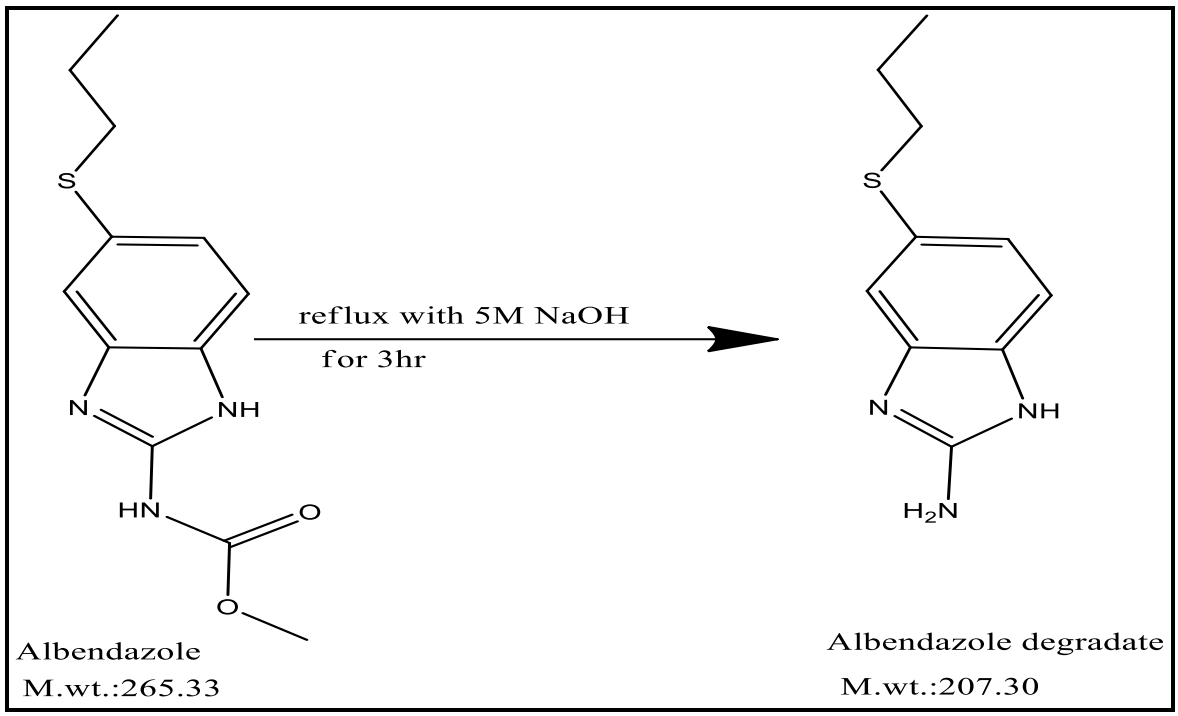

Figure 7. Proposed degradation pathway of ALB

Optimization of TLC-densitometric conditions

Initial trials on the cited drug and its degradation product were carried out to achieve good separation in which different developing systems with different ratios were tried, such as chloroform – methanol, chloroform – ethyl acetate, chloroform – ethyl acetate – acetic acid and chloroform – ethyl acetate – acetic acid – water. Best separation with almost well-defined spots was achieved using a mobile phase of dichloromethane– methanol (90:10, v/v). The selected mobile phase allows the determination of ALB without tailing of the separated band or interference provides better precision.

Different scanning wavelengths were tried such as 212, 220, 232 and 260 nm and it was found that, 232 nm wavelength was the most suitable for determination of ALB in the presence of its alkaline degradation product. The plates were visualized under UV lamp at 232 nm, where spots appear at Rf 0.63 for intact ALB and 0.27 for its alkaline degradate, **Figures 8, 9**.

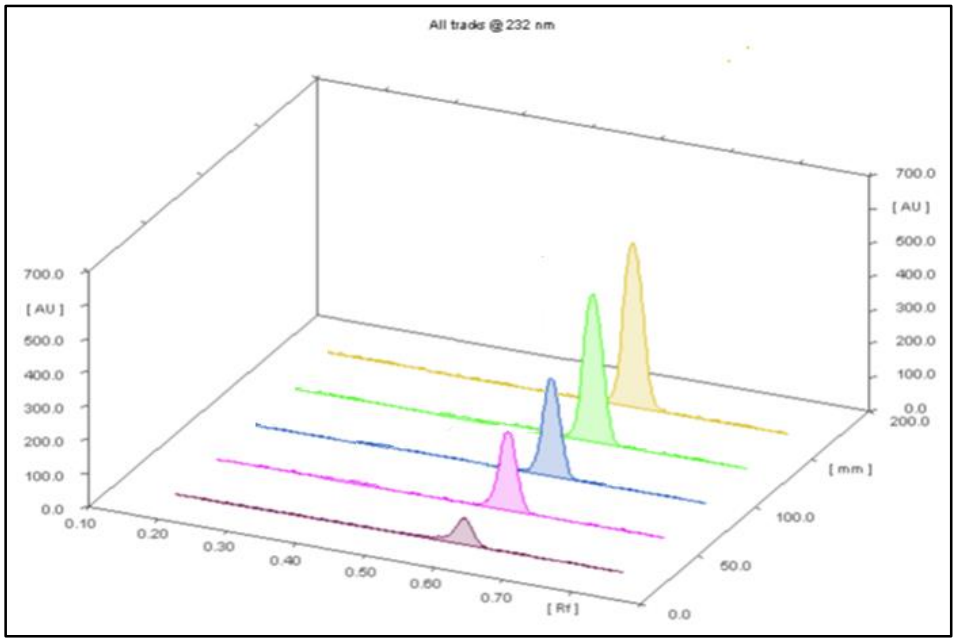

Figure 8. Densitometric chromatogram of ALB (1–5 µg/spot) at 232 nm.

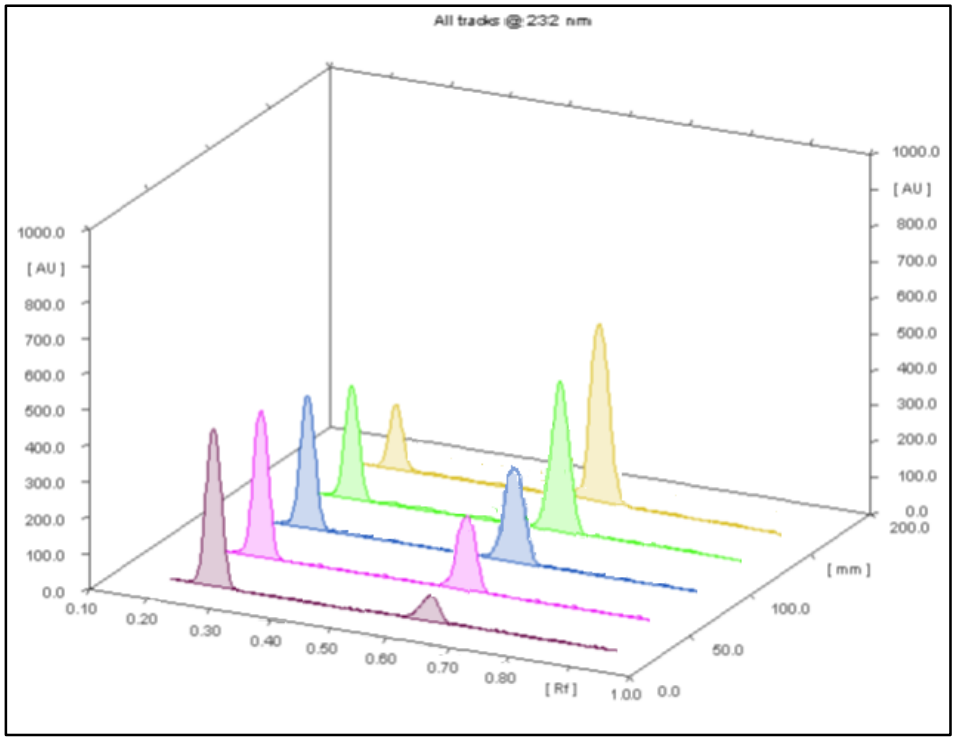

Figure 9. Densitometric chromatogram of ALB (5–1 µg/spot) and its alkaline degradation product (1–5 µg/spot) at 232 nm.

Chemometric methods

Due to the overlapped spectra of the drug and its degradate, **Figure 10**, the previous chemometric methods have been used to analyze this mixture. Various criteria have been developed to select the optimum number [26]. Thirteen samples (odd numbers of samples) were chosen and used for calibration and twelve (even numbers of samples) were used for external validation.

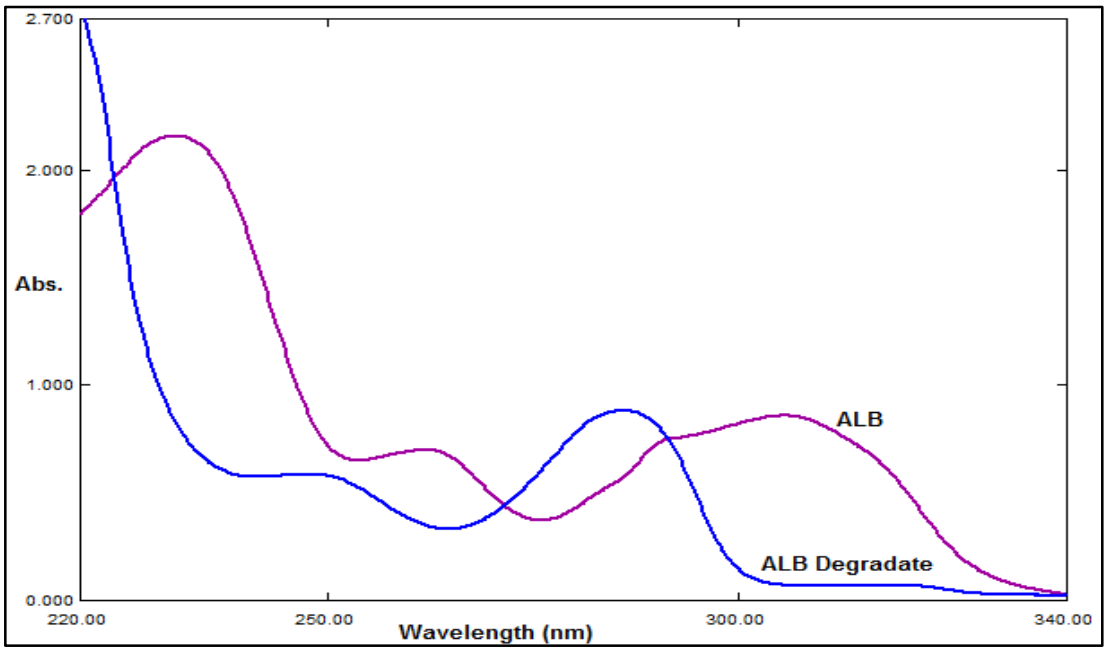

Figure 10. Absorption spectra of ALB (20 µg/ml) and its alkaline degradate (20 µg/ml)

For the CLS method, the training set was used for constructing CLS model or (K) matrix (i.e. absorptivity at different wavelengths) but poor predictions were obtained. The results were greatly improved by using the CLS model with nonzero intercept.

Cross-validation methods leaving out one sample at a time was employed. The predicted concentrations were compared with the known concentrations of the compounds in each calibration sample. The root mean squares error of cross-validation (RMSECV) was calculated for each method for examining the errors in the predicted concentrations. The optimum number of factors was selected by following the criterion of Haaland and Thomas [27]. The selected model was that with the smallest number of factors such that RMSECV for that model was not significantly greater than RMSECV from the model with additional factor. A number of factors were found to be optimum for the mixture of ALB and the degradation product using PCR and PLS, **Figure 11** and **Figure 12,** respectively.

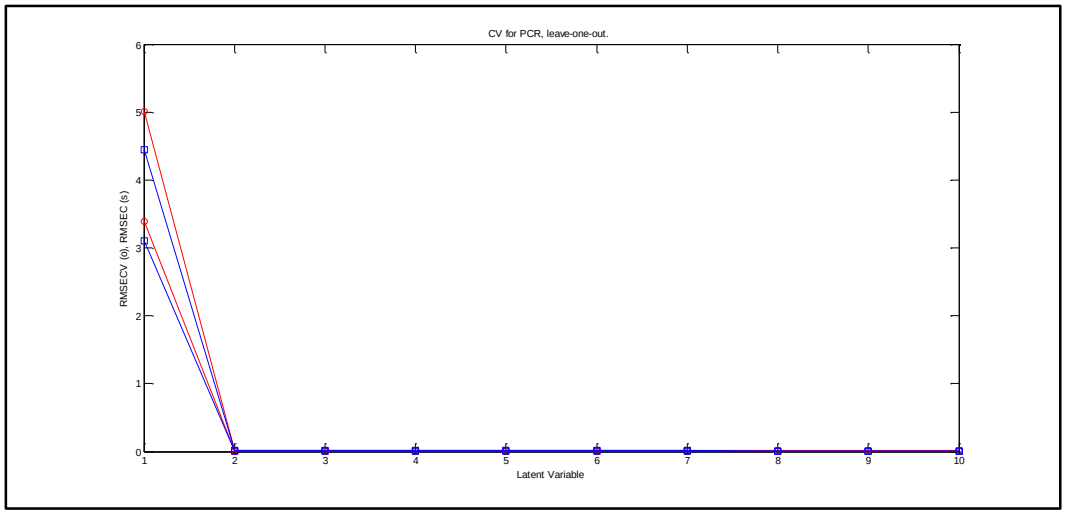

Figure 11. RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PCR model

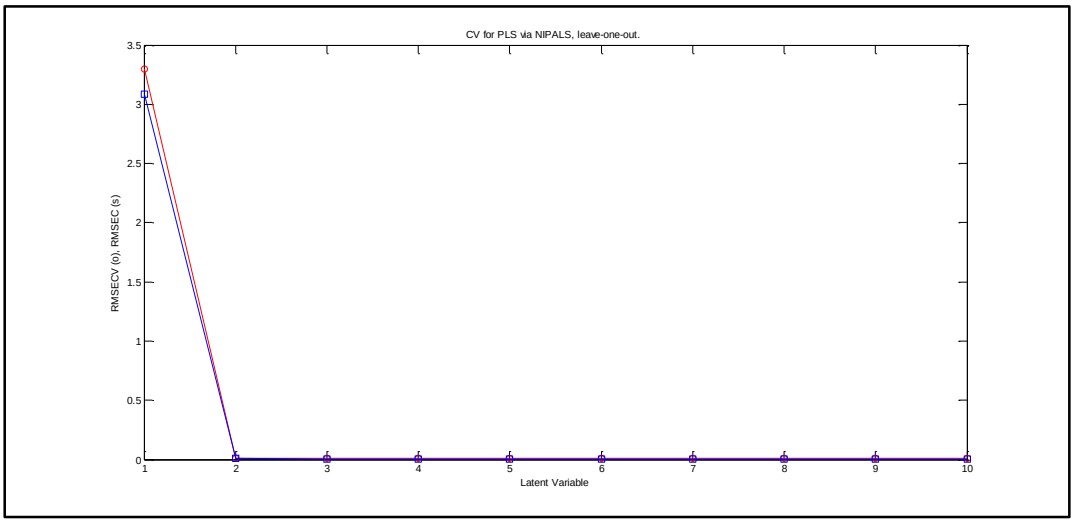

Figure 12. RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PLS model

The percentage recoveries of the validation samples are shown in **Table 2**, indicated the high predictive abilities of PCR, PLS and CLS models. When results obtained by applying the proposed methods for analysis of ALB and its degradation product compared to those obtained by applying the reported method [28], they showed no significant difference regarding accuracy and precision; and results were given in **Table 3.**

Table 2. % of ALB and its degradation product in the validation set by PCR, CLS and PLS methods

Validation mixture	CLS		PCS		PLS
	ALB	Degradate	ALB	Degradate	ALB
1	100.07	99.79	100.07	99.79	100.07
2	99.50	100.10	99.50	100.10	99.50
3	99.89	100.07	99.89	100.07	99.89
4	99.99	99.95	99.99	99.95	99.99
5	99.89	100.09	99.89	100.09	99.89
6	100.01	100.07	100.01	100.07	100.01
7	99.92	100.06	99.92	100.06	99.92
8	100.02	100.07	100.02	100.07	100.02
9	100.02	100.14	100.02	100.14	100.02
10	99.87	100.08	99.87	100.08	99.87
11	99.95	100.12	99.95	100.12	99.95
12	100.13	99.87	100.13	99.87	100.13
Mean+%RSD	9.94±0.159	100.03±0.106	99.93±0.159	100.03±0.107	99.93±0.158
RMSEP	0.012	0.014	0.013	0.015	0.012

Table 3. Determination of ALB in alzental ® tablets by the proposed methods and the reported method

Parameters	TLC-densitometry	CLS	PCR	PLS	Reported method [29]
N*	5	5	5	5	6
$\bar{X}$**	100.07	99.98	99.90	99.79	99.55
SD	0.840	0.295	0.747	0.565	0.533
%RSD	0.839	0.295	0.748	0.566	0.534
t***	1.198(1.833)	1.690(1.833)	0.878(1.833)	0.720(1.833)	
F***	2.484 (6.26)	3.264 (6.26)	1.964 (6.26)	1.124 (6.26)	

***** Number of experiments.

** The mean of percent recovery of pharmaceutical preparation.

***The values in parenthesis are tabulated values of "t "and"F" at(P = 0.05).

Pharmaceutical applications

For TLC-densitometric method

The proposed TLC-densitometric procedure was applied to the determination of ALB in alzental ® tablets. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives. The obtained results were statistically compared to those obtained by the reported method [28]. No significant differences were found by applying *t*-test and *F*-test at 95% confidence level [29], indicating good accuracy and precision of the proposed method for the analysis of the studied drug in its pharmaceutical dosage form, as shown in **Table 3.**

For chemometric methods

The suggested methods were valid and applicable for the analysis of ALB in alzental® tablets. The recovery percentages for ALB were found to be (100.29±1.002), (100.75±0.877) and (100.21±0.789) using CLS, PCR and PLS methods, respectively (average of 5 experiments) (**Table 3**).

Method validation: [for TLC-densitometric method] [30, 31]

Linearity and range

At the described wavelength, a linear relationship was found to exist between peak areas of the separated spots and the corresponding drug concentration over the range of 1–5 µg/spot, as shown in **Figure 13.**

$$y = 5839.7x - 674.1, \quad r^2 = 0.9997$$

where y is the peak area at 232 nm, x is the concentration of intact ALB in µg/spot, and r^2 is the squared correlation coefficient.

Linearity, range, regression equation, intercept, slope and squared correlation coefficient for the calibration data were presented in **Table 4**.

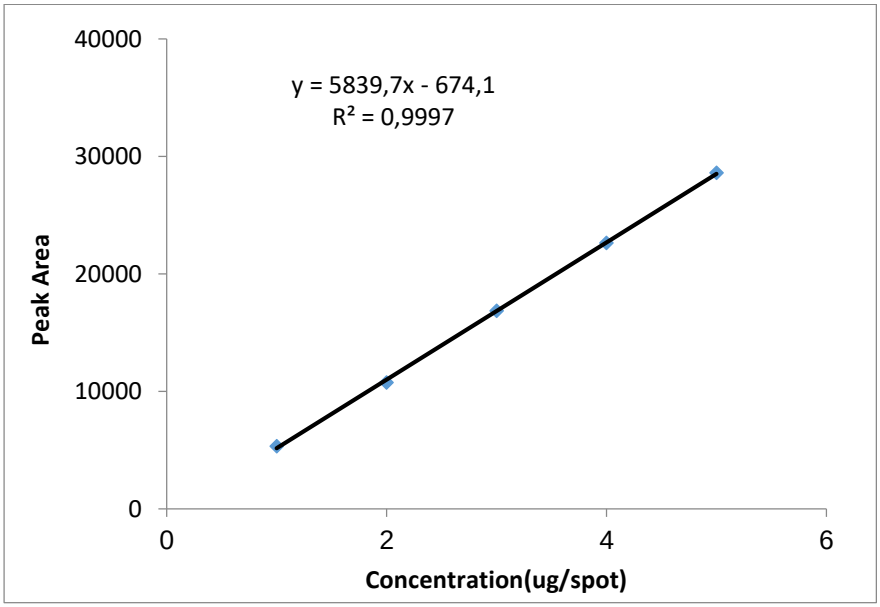

Figure 13. Calibration graph of ALB by the proposed TLC-densitometric method

Limits of detection and quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH guidelines from the following equations (2, 3):

$$\mathbf{LOD = 3.3\ S_a\ /\ slope} \qquad (2)$$

$$\mathbf{LOQ = 10\ S_a\ /\ slope} \quad (3)$$

where S_a is the standard deviation of y-intercepts of regression lines.

Results presented in **Table 4**, indicated that the method is sensitive for determination of the studied drugs.

Accuracy

The accuracy of the results was checked by applying the proposed methods for determination of different concentrations of ALB within the linearity range. The concentrations were obtained from the corresponding regression equations then the percentage recoveries were calculated, **Table 4**. To ascertain the accuracy of the suggested methods, recovery studies were carried out by standard addition technique at three different levels, **Table 5**.

Precision

The precision of the method was evaluated by calculating the relative standard deviation of the assay results. The mean relative standard deviations are presented in **Table 4** and can be considered to be satisfactory.

Table 4. Assay parameters and method validation for the determination of ALB by the proposed TLC-densitometric procedure

Parameters	Proposed TLC-densitometric procedure
λ_{max} (nm)	232
Linearity range (µg/spot)	1 – 5
LOD (µg/spot)	0.073
LOQ (µg/spot)	0.221
-Regression Equation	$y^* = b\,x^{**} + a$
- Slope	5839.7
- Intercept	674.1
Correlation coefficient (r^2)	0.9997
Accuracy (mean±S.D.)	99.95±0.322
Precision (%RSD)	
Repeatability	0.462
Intermediate precision	0.431

Table 5. Recovery study of ALB by adopting standard addition technique using the proposed TLC-densitometric procedure

Pharmaceutical taken (µg/spot)	Pure added (µg/spot)	Pure found (µg/spot)	Recovery %
1	2	1.99	99.57
	3	2.98	99.25
	4	3.95	98.83
Mean			99.22
RSD%			0.373

Table 6. Determination of ALB in mixtures with its degradate by the proposed TLC-densitometric procedure

Intact (µg/spot)	Degradate (µg/spot)	Degradate %	Intact found (µg/spot)	Recovery % of Intact
5	1	16.67	5.00	100.09
4	2	33.33	3.99	99.86
3	3	50	3.00	100.06
2	4	66.67	1.98	98.84
1	5	83.33	0.99	99.65
Mean				99.60
RSD%				0.536

Specificity

The specificity of the proposed procedure was assured by applying it to laboratory prepared mixtures of the intact drug together with its degradation product. The proposed procedure was adopted for the selective determination of intact ALB in presence of up to 83.33 % of its degradation product. The percentage recovery±RSD % was 99.60±0.536, as shown in **Table 6**.

Robustness

The robustness of the method was evaluated by slight changes in the chromatographic conditions such as mobile phase ratio (±1%), wavelength (±2nm.) and scanning speed (±1 mm/s). In each case only one parameter was changed while other conditions were kept constant.

The described minor changes did not affect the separation and resolution of ALB from its alkaline degradation product confirming robustness of the procedure.

Statistical analysis

Results obtained by the suggested methods for the determination of ALB in Alzental tablets were statistically compared with those obtained by applying the reported method [28]. The calculated t- and F-values were found to be less than the theoretical ones, confirming accuracy and precision at 95% confidence level [29], as shown in **Table 3**.

Another statistical comparison of the obtained results by the proposed methods and the reported method for the determination of ALB in pharmaceutical products using one-way ANOVA test was shown in **Table 7**. The results obtained by applying these methods show no significant differences between all of them. Moreover, the developed methods have the advantage of being more simple, rapid and economic over the reported ones.

Table 7. One-way ANOVA testing for the different proposed methods used for the determination of ALB in Alzental® Tablets

Drug	Source	DF	Sum of Squares	Mean Square	*F* value
ALB	Between exp.	4	1.745913	0.436478	1.144
	Within exp.	26	9.92018	0.381545	(2.743)

The values between parentheses are the theoretical F-values
The population means are not significantly different

CONCLUSION

The proposed methods are simple, sensitive, accurate and precise and can be used for the determination of ALB in pure form and in tablets (either alone or in the presence of its degradation product). These methods can be applied for the routine analysis of ALB in quality control laboratories. The developed chemometric methods have the advantages of being simpler and not expensive over the chromatographic method.

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