

Research Article



Development and Validation of a Spectrophotometric Method for Determination of Aliskiren in Tablets Using O-Phthalaldehyde

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ABSTRACT

Simple and rapid spectrophotometric method has been developed and validated for determination of aliskiren (ALS) in tablets. The proposed method based on the reaction of ALS with o-phthalaldehyde (OPA) and n-acetylcysteine (NAC) in a basic buffer. The measurement of absorbance was obtained at 335nm. Under optimized reaction conditions, Beer's law was obeyed in the concentration range 10-200 µg/ml. The regression equation for calibration data was: $y = 0.0102X + 0.0606$ with a correlation coefficient 0.9996. The molar absorptivity (ϵ) was $6.3 \times 10^5 \text{ L.mol}^{-1}.\text{cm}^{-1}$. The limit of detection (LOD) and limit of quantification (LOQ) were 2.8 and 8.5 µg/ml, respectively. The precision of the method was satisfactory; the values of relative standard deviation (RSD) did not exceed 2%. No interferences were observed from excipients that are present in the tablets. The proposed method was applied successfully for the determination of ALS in its pharmaceutical tablets with good accuracy and precision; the label claim percentage was $99.45 \pm 0.76\%$. The results were compared favorably with a reference pre-validated method. The method is practical and valuable in terms of its application in quality control laboratories.

Keywords: Aliskiren, O-phthalaldehyde, N-acetylcysteine, Spectrophotometry, Validation.

INTRODUCTION

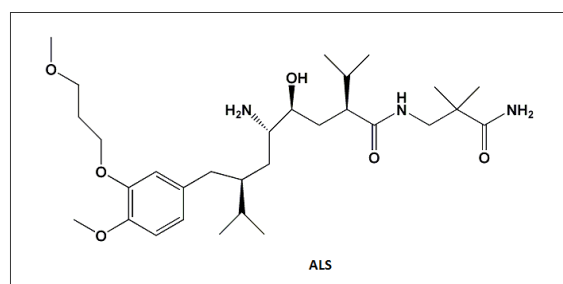
The importance of renin-angiotensin-aldosterone system (RAAS) in diseases such as hypertension, congestive heart failure and chronic renal failure has long ago been recognized and targeted for hypertension therapy. Renin inhibitors block the RAAS at its origin, reducing generation of angiotensin I and angiotensin II. Recent researches lead to discovery of several classes of nonpeptidic renin inhibitors¹⁻⁵. Aliskiren (ALS) (2(S),4(S),5(S),7(S)-N-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]-octanamide) (Scheme 1), is the first in a new class of orally active, non-peptide, low molecular weight renin inhibitors. ALS is used as a new effective treatment for hypertension⁶⁻⁸.

There is no official analytical method for analysis of ALS. The literature reveals various methods for the determination of ALS alone or in combination with other antihypertensive agents in pharmaceutical preparations. They include direct spectrophotometry^{9,10}, reverse phase HPLC methods¹¹⁻¹⁷ and MEKC method¹⁸. Two spectrofluorimetric assays of ALS after derivatization with dansyl chloride and fluorescamine in dosage forms and biological fluid were also described. The sensitivity was high and comparable with instrumental assays but required extraction of the product^{19,20}.

Spectrophotometry is considered as the most convenient analytical technique in many quality control laboratories, because of its wide availability, cost effectiveness, simple instrumentation and less time consuming. Furthermore, it does not need costly instrumentation and toxic solvents required for chromatographic methods. Although,

spectrophotometric analysis after chemical derivatization increases sensitivity and selectivity²¹, however up to now, no method of ALS determination via derivatization in dosage forms by spectrophotometry was reported.

O-Phthalaldehyde (OPA) a well-known derivatizing agent has been used in spectroscopic determination of pharmaceutical primary amines²²⁻²⁴. The reaction between ALS and OPA has not been investigated yet. Therefore, it was decided to apply this reaction and to develop a new simple and rapid spectrophotometric method for determination of ALS in dosage forms.



Scheme 1: Structure of Aliskiren.

MATERIALS AND METHODS

Instrumentation

SHIMADZU UV-1601 spectrophotometer and 1 cm quartz cells were used. Measurements of pH were made with WTWpH 526 Digital pH meter.

Materials

All chemicals used were of analytical grade. Aliskiren standard was kindly supplied by Novartis Pharmaceutical (Cairo-Egypt) and film coated tablets Rasilez® containing

300 mg ALS were obtained from a local pharmacy in (Darmstadt-Germany).

Preparation of reagents

OPA solution (0.05%): Prepared by dissolving 50 mg of OPA in 3 ml methanol and dilute with distilled water up to 100 ml. The solution was freshly prepared.

NAC solution (0.06%): Prepared by dissolving 60 mg of NAC in 100 ml volumetric flask with distilled water.

Borate buffer (0.2 M, pH 10.0): Prepared by dissolving 3.092 g boric acid in 250 ml with distilled water. Adjustment of pH was performed by 1 M sodium hydroxide.

Standard stock solution

100 mg of ALS base were accurately weighed and dissolved with distilled water in 100 ml volumetric flask to produce a solution having a concentration of (1mg/ml).

Procedure for calibration curve

Aliquots 0.1 – 2.0 ml of standard stock solution (1mg/ml) were transferred into a series of 10 ml volumetric flasks. A 1.0 ml of OPA (0.05%) and 1.0 ml NAC (0.06%) and 1.0 ml borate buffer (0.2 M, pH 10.0) were added, mixed gently and allowed to stand at room temperature for 10 min. The mixtures were diluted with distilled water and the absorption was measured at λ 335 nm against blank. The absorbances were measured and plotted against concentrations of ALS. The regression line and correlation coefficient were evaluated.

Procedure for ALS tablets

Ten Rasilez® tablets were weighed, crushed and their contents mixed thoroughly. An accurately weighed portion of powder equivalent to 100 mg ALS was transferred into 100 ml volumetric flask. About 75 ml of distilled water was added and extraction was performed mechanically for 1 hr. The volume was brought to 100 ml with water and final solution was filtered. Aliquot from the solution covering the working concentration range was transferred in 10 ml volumetric flask and analyzed as described under Procedure for calibration curve. The results were compared with a reference method statistically.

RESULTS AND DISCUSSION

Absorption spectra

Determination in pharmaceutical preparation based on direct measurement of absorption is susceptible to potential interferences. ALS exhibits λ_{max} at 279 nm. Derivatization of ALS to produce a red shifted derivative can increase both selectivity and sensitivity of a spectrophotometric assay. The presence of primary amine group in ALS enabled chemical derivatization with OPA. OPA reacts with amines rapidly at room temperature in an aqueous basic medium and in the presence of a mercaptan (RSH) to form an absorbing and fluorescent isoindole derivatives²⁵. The resulting

chromogen exhibits a maximum absorption peak (λ_{max}) at 335 nm (Figure 1). This wavelength has been used for measuring the absorbance of the derivatives, which were soluble in water.

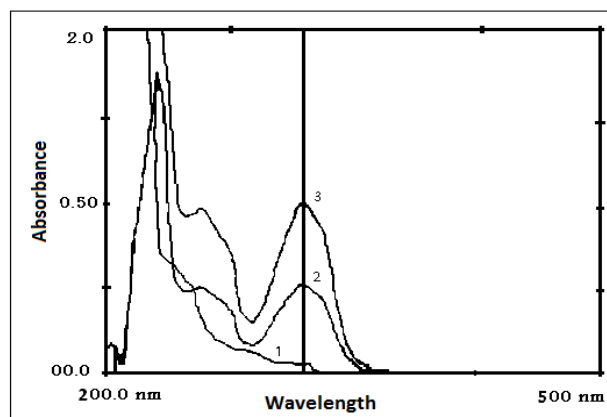


Figure 1: Absorption spectra: (1) blank spectrum against water; (2) and (3) the reaction product of ALS 50 and 100 µg/ml with OPA-NAC against blank, respectively.

Optimization of reaction variables

Effect of OPA concentration:

To Study the effect of OPA concentration on its reaction with ALS, different OPA solutions (0.02 - 1.7%) were used according to the procedure of calibration curve and the absorbances were measured. When OPA concentration ranged from 0.05 to 1.5%, the changes in absorption readings were non-significant. A higher OPA concentration decreased the absorption slightly. The highest reading was obtained at a concentration of 0.05% and the optimum volume of this solution was 1.0 ml.

Effect of NAC concentration:

OPA reacts only with primary amines in the presence of SH-donor compound. NAC was applied in the study since it is advantageous regarding the stability of its adduct with OPA/primary amine²⁵⁻²⁸. In addition, NAC lacks the unpleasant odor of other free thiols. Different NAC concentrations (0.02 – 2.0 %) were tested. It was found, a 1.0 ml of NAC in a concentration of 0.06% was sufficient to give maximum absorbance, despite of increasing the amount of NAC up to 2.0%.

Effect of OPA : NAC mole ratio:

Changing the molar ratio of OPA to NAC additive (1:0.3 - 1:3) was studied at ALS concentration 100 µg/ml. A 1:1 molar ratio (Figure 2) was satisfactory and resulted in the highest absorbance intensity.

Effect of ALS : OPA mole ratio:

The molar ratio of ALS to OPA was changed within the range (0.05: 1 - 2.5:1) at NAC concentration 0.3 %. The maximum absorbance was at 1:2 molar ratio (Figure 2).

Effect of pH:

The effect of pH on absorption of reaction product was examined using bicarbonate and borate buffers over pH

range 9.2 -11.0, since OPA reacts under alkaline conditions. The maximum absorption was achieved when the reaction was carried out with borate buffer pH 10.0. The optimum volume of this buffer was 1.0 ml.

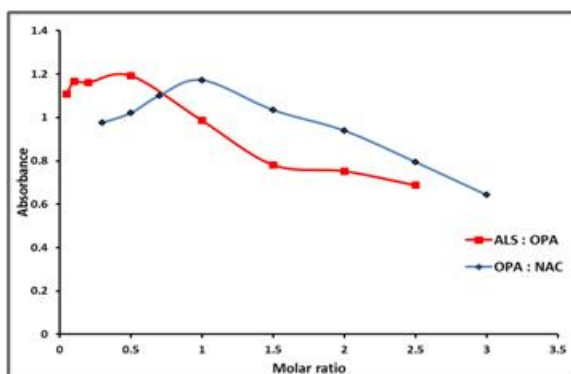


Figure 2: Effect of ALS: OPA and OPA: NAC mole ratio on derivatization reaction.

Effect of temperature and time:

Temperature had a negative effect on absorption of the reaction. The reaction goes to completion within 5 min. at room temperature ($25 \pm 2^\circ\text{C}$). Elevated temperature up to 60°C decreased the absorption. This was probably attributed to the instability of ALS-OPA-NAC product. To establish simple and reproducible procedure further experiments were carried out at room temperature for 10 min. Derivatization using OPA is characterized by simplicity and rapidity with no need for extra equipment (water bath).

Effect of diluting solvent:

Upon diluting the reaction solution with water, a transparent solution was obtained indicating the solubility of ALS-OPA-NAC derivative. In order to select the most appropriate solvent for diluting the reaction solution, different solvents were used and the absorption values were recorded. These solvents were methanol, ethanol, isopropanol, acetone, and acetonitrile. Absorption values were slightly influenced. Water was used as a diluting solvent in the experiments to avoid exposure to toxic organic solvents and to lower the analysis cost.

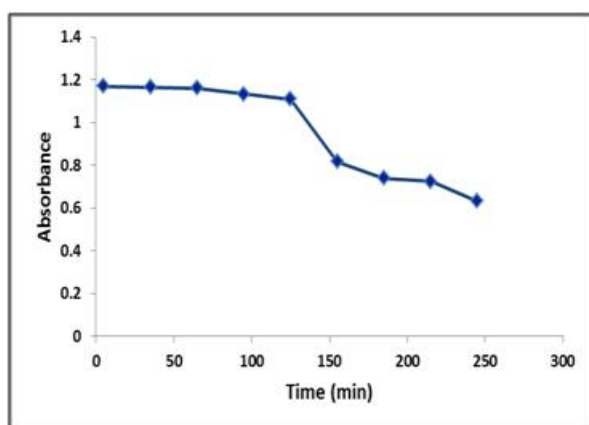


Figure 3: Stability of ALS-OPA-NAC chromogen.

Stability of ALS-OPA-NAC chromogen:

Under the aforementioned optimum conditions, the effect of time on the stability of the chromogen was studied by following the absorption values of the reaction solution after dilution at different time intervals (up to 4 hrs). It was found that the absorbance of the chromogen remains stable for at least 90 min (Figure 3). This increased the convenience of the method as well as making it applicable for a large number of samples.

Validation of the method:

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines²⁹.

Calibration and sensitivity:

Under the optimum reaction conditions the calibration curve for the determination of ALS by its reaction with OPA and NAC was constructed by plotting the absorbances as a function of the corresponding concentrations. The regression equation was $Y = 0.0102 X + 0.0606$ ($r = 0.9996$), where Y is the absorbance at 335 nm and X is the concentration of ALS in $\mu\text{g/ml}$ in the range 10-200 $\mu\text{g/ml}$, and r is the correlation coefficient. The molar absorptivity (ϵ) was $6.3 \times 10^5 \text{ L.mol}^{-1}.\text{cm}^{-1}$. The limit of detection (LOD) and limit of quantification (LOQ) were determined using the formula: $\text{LOD or LOQ} = \kappa \text{SDa/b}$, where $\kappa = 3$ for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope. The LOD and LOQ were found to be 2.8 and 8.5 $\mu\text{g/ml}$, respectively.

Accuracy and precision:

The accuracy and precision of the method were evaluated by analyzing three different concentrations of pure drug solution (50, 100, 150 $\mu\text{g/ml}$) and each determination was repeated three times. The relative error (% RE) was less than 2% indicating the accuracy of the analytical procedure (Table 1).

For intra- and inter-day precision the relative standard deviation (RSD) values were in the range 0.52 - 1.42 % (Table 1) indicating good precision of the proposed method. This precision level is adequate for routine analysis of ALS in quality control laboratories.

Specificity:

The specificity of the method was evaluated by investigating the interference liabilities from common excipients that might be added during pharmaceutical formulation. Known amount of ALS (100 mg) was mixed with different levels of excipients (Table 2) in 100 ml volumetric flask and analyzed by the proposed method. The average recovery value was $100.2 \pm 0.28\%$ confirming the absence of interferences from any of the common excipients with the determination of ALS by the proposed method.

Table 1: Evaluation of intra-day and inter-day accuracy and precision.

Concentration µg/ml	Intra-day n = 5		Inter-day n = 6	
	%RE	%RSD	%RE	%RSD
50	0.48	1.16	0.61	1.42
100	0.31	0.57	0.43	0.81
150	0.23	0.77	0.37	0.62

RE: Relative error, RSD: Relative standard deviation.

Table 2: Specificity of the proposed method for ALS determination.

Excipient ^a	Recovery (% ± SD) ^b
Starch (50 mg)	99.53 ± 0.21
Glucose (50 mg)	99.32 ± 0.11
Sucrose (50 mg)	100.2 ± 0.53
Talc (50 mg)	101.7 ± 0.22
Povidone (10 mg)	98.97 ± 0.16
Crospovidone (10 mg)	99.92 ± 0.41
Magnesium stearate (10 mg)	101.5 ± 0.3
Average ± SD	100.2 ± 0.28

^a: Amount of excipient mixed with 100 mg ALS, ^b: Values were mean of three determinations, SD: Standard deviation.**Robustness and ruggedness:**

Robustness was examined by evaluating the influence of small variation in the method variables including concentration of reagents, volume of reagents and reaction time on its analytical performance. In these experiments one parameter was changed whereas the others were kept unchanged and the recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the procedures; recovery values were 98.7 – 100.49 ± 0.082 – 1.049% (Table 3). This indicated the reliability of the proposed method during its routine application for the analysis of ALS.

Ruggedness was assessed by using the same operational conditions in two different laboratories with different instruments at different elapsed times. Results obtained from lab-to-lab and day-to-day variations were reproducible as the RSD did not exceed 2%.

Application of the proposed method to analysis of ALS in tablets

It is evident from the above mentioned results that the proposed method gave satisfactory results with ALS in Bulk. Thus, its tablets Raseliz[®] were subjected to analysis of their ALS content by the developed method and a reference method⁹. The label claim percentage was 99.45 ± 0.76 %. This result was compared with that obtained from the reference method by statistical analysis with respect to the accuracy by *t*-test and precision by *F*-test

(Table 4). No significant difference was found at 95% confidence level providing similar accuracy and precision in the determination of ALS by both methods.

Table 3: Robustness of the proposed method

Recommended condition	Parameter	Recovery (% ± SD) ^a
OPA concentration (% w/v)	0.04	98.87 ± 0.19
	0.06	100.49 ± 0.602
OPA volume (ml)	0.8	98.95 ± 0.287
	1.2	99.77 ± 1.049
NAC concentration (% w/v)	0.05	98.70 ± 0.246
	0.07	98.83 ± 0.441
NAC volume (ml)	0.8	98.82 ± 0.135
	1.2	98.94 ± 0.104
0.2 M Borate buffer (pH)	9.8	100.08 ± 0.759
	10.2	99.87 ± 0.953
Time of the reaction (min)	8	98.74 ± 0.082
	12	99.19 ± 0.316

^a: Values were the mean of three determinations, SD: Standard deviation.**Table 4:** Determination of ALS in tablets by the proposed method and a reference⁹.

Dosage form	Recovery (% ± SD) ^a		t-value ^b	f-value ^b
	Proposed method	Reference method		
Raseliz [®] (300 mg)	99.45 ± 0.76	100.76 ± 0.83	1.21	1.09

^a: Values were mean of five determinations, ^b: The tabulated values for *t* and *f* at 95% confidence limit are 2.78 and 6.26, respectively.**CONCLUSION**

The present study described, for the first time, the successful application and evaluation of OPA/NAC derivatization method for simple, rapid and specific spectrophotometric analysis of ALS. The proposed method has the advantage of being sensitive and avoiding the use of expensive and sophisticated instruments, as well as the availability of these reagents in any analytical laboratory. Therefore, the developed method is suitable and practical for routine analysis of ALS in quality control laboratories.

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