Method development and validation of aliskiren in tablet formulation by UV spectrophotometric methods

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Abstract
Optical methods through ultraviolet-spectrophotometer were developed and justification was done to validate the method for quantitative determination of aliskiren in its tablet formulation. Wavelengths for different methods were chosen by running selected sample in spectrum mode of the instrument, it was 279 nm; absorbance maxima for zero order technique, 289 nm; peak minima for first order derivative technique and between 269 nm and 289 nm for AUC technique using distilled water as reference. After studying linearity graph, working concentration range selected for method I, method II and method III was in between 25 to 150 µg mL^{-1}. R2 value equal to 0.999 confirmed a better correlation between concentration and absorbance. Percentage purity of aliskiren in tablet formulation was found in between 98.54-100.36 which was very close to label claim. Validation following ICH guidelines was done to justify the results obtained through method I, method II and method III. The percentage of the standard drug recovered in recovery study for all the three techniques were within the limit and were in between 98.61-101.66. The percentage relative standard deviation in precision study was less than 2 for all the techniques. From the observed data it can be said that the developed methods can be utilized for quantitative estimation of aliskiren in tablet formulation.

Keywords: Ultraviolet-Spectrophotometer, Optical methods, Aliskiren, Validation, First order derivative, AUC.

Introduction
Hypertension is a common disease that causes cardiovascular problems and even death in world wide. Control in BP is essential to manage cardiovascular problems and their complications1. Aliskiren hemifumarate inhibit rennin secretion and play an important role in management of hypertension. The new antihypertensive agent Aliskiren hemifumarate has potential to effectively control blood pressure2.

Aliskiren hemifumarate (ALH) is chemically (2S, 4S, 5S, 7S)-N- (2-methylpropyl) 5amino-4-hydroxy-2, 7 disopropyl-8-[4-methoxy-3-(3-methoxypropoxy)-phenyl]- octamide hemifumarate (Fig. 1). ALH is white to slightly yellowish in colour, it exhist in form of crystalline powder. Molecular weight of ALH is 609.8 while molecular weight of its free base is 551.8. It is soluble in water, ethanol, DMSO, phosphate buffer and in n-octanol. Melting point of the drug ranges from 99-105 ^{o}C. It is the first drug of this category emerged as a new class of antihypertensive agents. ALH blocks the renin system at its rate-limiting step by directly inhibiting the catalytic activity of renin, thereby reducing generation of angiotensin I and angiotensin II 3, 4.

Literature review 5-8 confirmed that there are few methods reported for the detection of ALH in tablet dosage form by ultra violet- spectrophotometry and RP-HPLC. From the literature it was observed that distilled water can be used instead of methanol to reduce the cost of methods and can be made simpler in comparison to reported methods. It is essential to continuously develop new, simple, accurate and economical methods through UV spectroscopy so that better option remains available for estimation of coming drugs. The objective of the present research work is to develop economical UV spectroscopic methods for estimation of Aliskiren (ALS) in tablet dosage form for routine analysis with reduced cost. To validate the developed methods ICH guidelines[9] were followed.

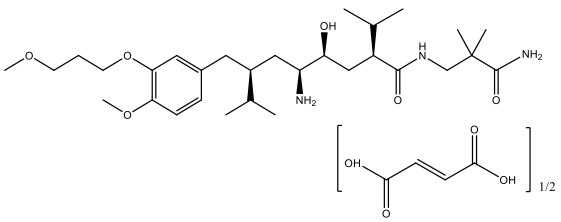

Fig. 1: Structure of Aliskiren Hemifumarate

Materials and Method
Reagents
Pure drug, Aliskiren hemifumarate was collected from Swapnroop Pharmaceuticals, Aurangabad, Maharashtra, India. Rasilez tablet label claimed 150 mg was purchased from drug store of local market. Distilled water was

utilized as solvent in developing methods.

Instrumentation

Shimadzu 1800 double beam UV-VIS spectrophotometer (Japan) with a resolution of 1 nm, having wavelength Range of 190- 1100 nm was used. Users can find data on a PC using software UV Probe (version 2.31). 10 mm sample cell made of quartz was used. Electronic balance (Schimadzu - 220h) having sensitivity of 0.001 g was used for weighing.

Method development

Preparation of stock solution of pure drug

Pure drug stock solution of Aliskiren (ALS) was prepared by weighing accurately aliskiren hemifumarate equivalent to 100 mg of ALS in 100 mL graduated flask, small amount of distilled water was added o dissolve the drug completely and volume was made up to the mark with the selected solvent. To prepare working standard solution of 50µg mL^{-1}, 2.5 ml stock solution was pipette out and transferred into a 50 mL graduated flask, volume was made up to the mark with distilled water. From the stock solution different desired concentrations were prepared by transferring appropriate volume in different graduated flask.

Plotting of linearity graph

Method I: zero order ultra violet spectroscopic method

The standard solution (50µg mL^{-1}) of ALS in distilled water was taken to scan in spectrum mode selecting ultra violet wavelength range. The UV spectrum showed that sample had maximum peak at 279 nm (Fig. 2). This wavelength was selected for further measurements. The calibration curve was plotted (concentration corresponding to absorbance) to find out the linearity of solution. The graph was found to be linear over a concentration range of 25-150 µg mL^{-1}. Linearity was observed through overlain spectra of various concentrations of ALS (Fig. 3). The R^2 value was calculated and analyzed to find out regression coefficient.

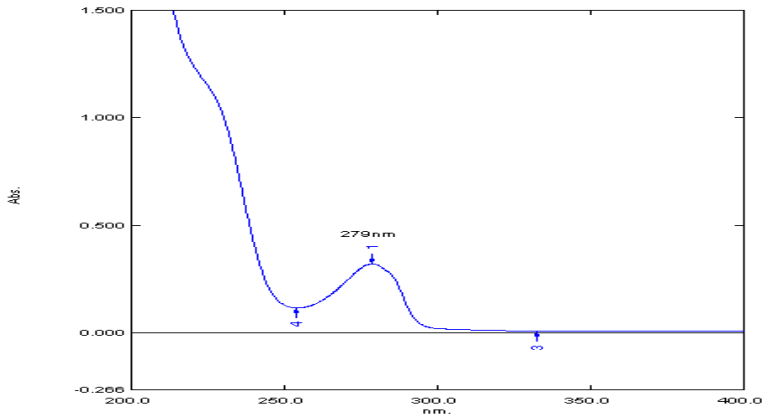

Fig. 2: Zero order UV-spectra showing λ_{max} of Aliskiren

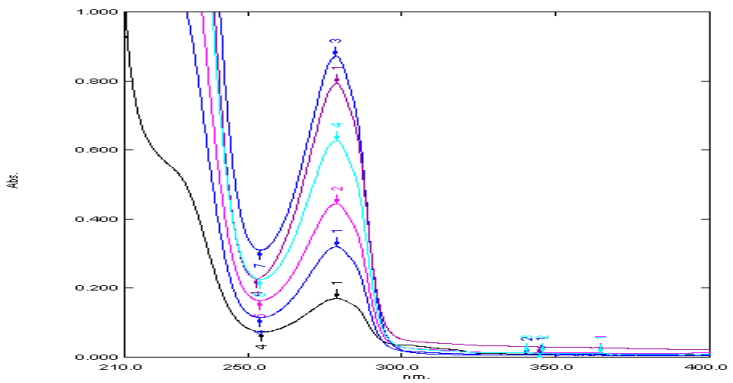

Fig. 3: Overlay spectra representing different concentrations of Aliskiren

Method II: first order derivative ultra violet-spectroscopic method

Transformed zero order basic spectra into first order derivative with the help of software at wavelength interval of 2 ($\Delta\lambda = 2$) and scaling factor taken as 1. The response ($dA/d\lambda$) from the recorded spectra was measured at peak minima of 289 nm (Fig. 4). To find out linearity and working concentration range a calibration graph (concentration corresponding to response $dA/d\lambda$) was drawn. Linearity range and R^2 value was obtained by analysing graph and regression equation respectively.

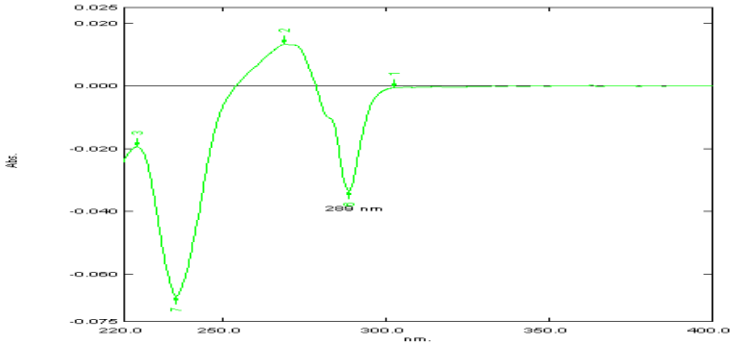

Fig. 4: First order derivative ultra violet-spectra of Aliskiren

Method III: Area under curve method

It involves measurement of AUC between two selected wavelengths λ_1 and λ_2.Several trials were done to select the wavelength range to obtain the linearity between AUC and concentration. After selection of wavelength range zero order spectra of different concentration was transformed in area under curve mode. Area was recorded between 269 nm and 289 nm (Fig. 5). To obtain the linearity a calibration graph (AUC in corresponding to concentration) was prepared. Linearity range and R^2 value was obtained by analyzing regression equation respectively.

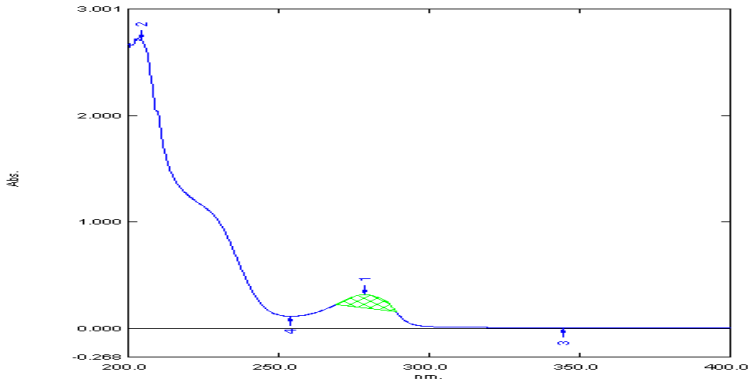

Fig. 5: Area under curve ultra violet -spectra of Aliskiren

Quantitative estimation of aliskiren in tablet dosage form

Preparation of sample solution

To prepare sample solution of ALS accurately weighed twenty tablets (label claimed 150 mg) and mean of weight of twenty tablets was calculated. The tablets were crushed with mortar and pestle and the powder equivalent to 100 mg of ALS was accurately weighed and transferred into a 100 mL graduated flask. Solvent was added into graduated flask, prepared solution was sonicated, filtered through Whatman filter paper No.41 and was made up to the mark with distilled water. Stock solution was further diluted to get required concentration of 50µg mL^{-1}separately in different graduated flasks. The concentration of ALS in formulation was determined by above developed methods. Assay procedure was repeated six times for each method.

Method Validation: Validation of the methods was carried ut by following ICH guidelines[9] to study various validation parameters.

Linearity: To find out working concentration range of ALS, linearity graph was prepared for all the developed spectroscopic methods. For all the methods it was observed linear in the concentration range of 25-150 µg mL^{-1}. A good linear relationship (R^2=0.999) was observed between the concentrations of ALS and the corresponding absorbance. The regression analysis was performed to obtain analysis results.

Accuracy: Recovery of standard solution was measured by adding standard solutions of ALS at 80%, 100% and 120% levels to previously determined tablet sample solutions. Total amount was calculated from the absorbance values recorded and percentage recovery of standard solution was determined by subtracting total absorbance from predetermined sample absorbance. The data is given in terms of % recovery.

Precision: Closeness of measured value was confirmed by recording absorbance of samples at different time in same day (Intra-day) and at different days (Inter-day). To find out the intra-day and inter-day precision of the methods, absorbance values of ALS solution of three different concentrations were prepared and measured by all the three developed ultra violet-spectrophotometric methods of analysis.

Percentage relative standard deviation was calculated for each concentration level for each method of intra-day and inter-day precision.

Results and Discussion

Three ultra violet-spectrophotometric methods were selected for quantitative estimation of ALS, Zero order as Method I, First order derivative as Method II and Area under Curve as Method III. The absorption maxima for method I was found to be 279 nm (Fig. 2). Linearity was confirmed by recording overlain spectra of various concentrations of ALS (Fig. 3). Wavelength of peak minima selected for quantitative analysis through derivative method was 289 nm (Fig. 4). 269-289 nm; working wavelength range for AUC method was selected (Fig. 5). Working linearity range was decided by making different concentrations of solution and measuring their response through developed methods. A broad working concentration range of 25-150 µg/mL for all the developed methods were finalized as reported in Table 1. The % assay by the three developed methods was found in between 98.54-100.38 as mentioned in Table 2. Percentage recovered amount of pure drug was in between 98.58-99.19 for method I, 98.88-101.23 for method II and 99.96-101.34 for method III, which confirmed there is no interference of sample and its diluents in recovery of pure drug as shown in Table 3. The methods were precise as percentage relative standard deviation was within the limit of ± 2% as shown in Table 4. Considering all the parameters of validation studies and % assay it was suggested that spectroscopic methods can be applied for quantitative estimation of ALS in tablet formulation.

Table 1: Linearity Study by Developed Methods

Parameter	Method I	Method II	Method III
Wavelength	279	289	269-289
Linearity Range (µg/ml)	25-150	25-150	25-150
Regression equation (Y = mx+c)	y = 0.006x + 0.002	y = 0.0006x - 0.001	y = 0.034x - 0.104
Correlation coefficient (r^2)	0.999	0.999	0.999
Slope (m)	0.006	0.0006	0.034
Intercept (c)	0.002	0.001	0.104

Table 2: Assay Results of Aliskiren by Developed Methods

Type of Method	Label claim (mg/tablet)	% Label claim*	SD	% RSD
Method I	150	98.54	±0.50	0.50
Method II	150	100.32	±1.35	1.35
Method III	150	100.38	±0.53	0.53

*Average of six determination

Table 3: Accuracy Study of Aliskiren by Developed Methods

Sample Conc. (µg mL^{-1})	% Level of standard	Conc. of standard added (µg mL^{-1})	*Amount recovered (µg mL^{-1})			% Recovery		
			I	II	III	I	II	III
50	80	40	88.72	91.11	89.97	98.58	101.23	99.97
50	100	50	99.16	98.88	101.34	99.16	98.88	101.34
50	120	60	109.11	110.55	110.17	99.19	100.50	100.16

*average of three determination

Table 4: Precision Studies of Aliskiren by Proposed Methods

Conc. (µg mL^{-1})	Intra-day precision					
	Method I		Method II		Method III	
	mean ±SD (n=6)	% RSD	mean ±SD (n=6)	% RSD	mean ±SD	% RSD
50	0.296±0.004	1.61	0.029±0.0005	1.74	1.62±0.002	0.16
75	0.460±0.001	0.30	0.044±0.0007	1.67	2.51±0.005	0.20
100	0.613±0.003	0.61	0.059±0.0009	1.64	3.33±0.008	0.26
	Inter-day precision					
	Method I		Method II		Method III	
50	0.299±0.004	1.53	0.029±0.0004	1.36	1.64 ±0.026	1.60
75	0.462±0.003	0.65	0.045±0.0008	1.80	2.51±0.017	0.71
100	0.611±0.004	0.79	0.061±0.0007	1.23	3.33±0.013	0.40

Conclusion

The three ultra violet-spectrophotometric methods were developed and validation was carried out following ICH guidelines for justification of methods. All the samples were prepared in distilled water to record the absorbance. By using distilled water as solvent total cost of the methods were reduced. Analytical work can be performed in broad concentration linearity range and shows good agreement between absorbance and concentration. Variations in form of % RSD of ± 2 indicate a high degree of precision of methods. Percentage recovered amount within ± 2% of standard value depict the methods to be accurate and reproducible. On the basis of these results conclusion was made that developed ultra violet -spectrophotometric methods can be utilized for the quantitative estimation of ALS in formulation in routine analysis.

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