ISSN- 2231–5667 (Print) www.asianpharmaonline.org
ISSN- 2231–5675 (Online)

RESEARCH ARTICLE

Development and Validation of Spectrophotometric Method for Determination of Aliskiren, Amlodipine and Hydrochlorothiazide in combined Pharmaceutical Dosage Forms

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ABSTRACT:
A simple, accurate, precise, economical and reproducible method was developed for simultaneous estimation of aliskiren, amlodipine and hydrochlorothiazide in Combined Pharmaceutical Dosage Forms. The excipients in the commercial tablet preparation did not interfere with the assay. The λmax for aliskiren, amlodipine and hydrochlorothiazide were 252 nm, 360 nm and 271 nm respectively. At 360 nm, Amlodipine showed some absorbance while aliskiren and hydrochlorothiazide showed zero absorbance so that amlodipine was estimated at 360 nm. While at 252 nm and 271 nm aliskiren and hydrochlorothiazide were determine by simultaneous estimation method after eliminating the absorbent of Amlodipine at this wavelength. Linearity in concentration range of 4-28 µg/mL, 4-28 µg/mL and 20 - 120 µg/mL with The mean recoveries were 99.97 ± 0.82, 99.93 ± 0.88 and 100.14 ± 0.81 % for ALK, AML and HTZ, respectively. Validation of the proposed method was carried out according to ICH guidelines. Thus the present study gives an excellent method for the determination of all the three drugs in combined dosage formulation without their prior separation

KEYWORDS: Amlodipine besylate, hydrochlorothiazide, Aliskiren, Spectrophotometric method.

INTRODUCTION:
Aliskiren, (2(S), 4(S), 5(S), 7(S)-N- (2-carbamoyl- 2-methylpropyl) -5- amino-4-hydroxy2,7 diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy) phenyl] octanamide hemifumarate) 1-3 (Fig.1).The first oral direct rennin inhibitor approved for clinical use, exhibits a novel and advantageous pharmacokinetic and pharmacodynamic profile for the long-term treatment of hypertension. Aliskiren blocks the renin system at its rate-limiting step by directly inhibiting the catalytic activity of renin, thereby reducing generation of angiotensin I and angiotens in II[1]. Hydrochlorothiazide 6-chloro-1, 1-dichloro-3, 4, dihydro - 2H-1, 2, 4-benzoliadiazine-7-sulphanomide1, 1-dioxide (fig. 2). belongs to Thiazide class of diuretics, acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubule. This increases the osmolarity in the lumen, causing less water to be reabsorbed by the collecting ducts. This leads to increase urinary output [2].

Amlodipine (AM), 2[(2-aminoethoxy) methyl]-4-(2-chloro-phenyl)-1, 4-dihydro-6-methyl-3, 5-pyridine carboxylic acid, 3-ethyl, 5-methylester (Fig.3) is a dihydro pyridine derivative with calcium antagonist activity. It is used in the management of hypertension, chronic stable angina pectoris and prinzmetal variant angina. Amlodipine inhibits the trans-membrane influx of calcium ions into vascular smooth muscle and cardiac muscle [3] .Literature survey revealed HPLC[4], RP-HPLC[5-7], simultaneous UVspectrophotometric [8] and spectroflurimetric[9] methods are reported for the estimation of aliskiren hemifumarate alone or in combination with other anti-hypertensive agents. Methods such as HPLC[10-14], RP-HPLC[15-17], LC-MS[18], UPLC[19]and simultaneous UV- spectrophotometric methods[20] are reported for the estimation of HCTZ alone or in combination with other anti-hypertensive agents. Methods such as HPLC[21], RP-HPLC[22-26], LC-MS[27] and simultaneous UV-spectrophotometric methods[28] are reported for estimation of amlodipine alone or in combination with other agents. As no spectroscopy method is reported for Aliskiren, Amlodipine and hydrochlorothiazide in

Received on 10.11.2014 Accepted on 29.11.2014

combination, the aim of the present study was to develop accurate, precise and selective reverse phase spectroscopy method assay procedure for the analysis of Aliskiren, Amlodipine and hydrochlorothiazide in combined dosage formula.

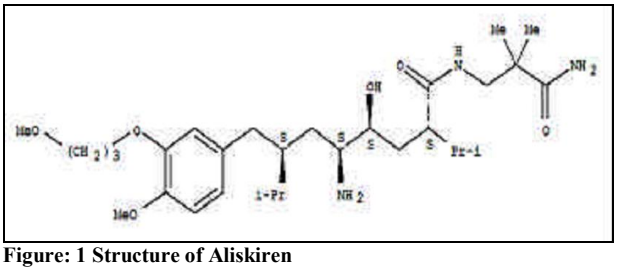

Figure: 1 Structure of Aliskiren

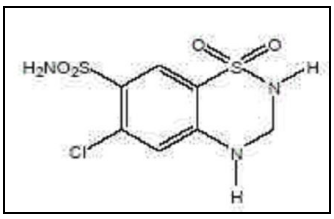

Figure: 2 Structure of Hydrochlorothiazide

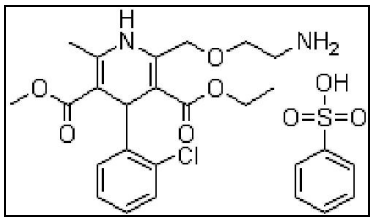

Figure: 3 Structure of Amlodipine

EXPERIMENTAL:

Apparatus

A double beam UV-visible Spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UV probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells, Sartorius CP224S analytical balance (Shimadzu, Japan), Ultra sonic cleaner (Life Care Eq. Pvt. Ltd, Mumbai, India), Corning volumetric flasks, pipettes of borosilicate glass were used in the study.

Reagents and Materials

Pharmaceutical grade of Aliskiren (ALK) was kindly supplied as a gift samples from Novartis India limited, India. Hydrochlorothiazide (HTZ) and Amlodipine besylate were kindly supplied as a gift samples from Torrent research centre, Gujarat (India), The pharmaceutical formulation (Amturnide) containing 150 mg ALK, 5 mg AML and 12.5 HTZ was procured from the local pharmacy, AR grade Methanol (S.D. Fine Chemical Ltd., Mumbai, India), Whatman filter paper no. 41 (Whatman International Ltd., England)

Preparation of Standard Solutions

Preparation of ALK, HTZ and AML Standard Solutions

Accurately weighed portions of ALK (100 mg), Amlodipine besylate (13.86 mg, which is equivalent to AML 10 mg) and HTZ (10 mg) were transferred to a separate 100 mL volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentrations of AKL (1000 µg/mL), AML (100 µg/mL) and HTZ (100 µg/mL).

Preparation of Sample Solutions

Twenty tablets were weighed and powdered. The quantity of the powder equivalent to 30 mg of ALK, 1.0 mg of AML and 2.5 HTZ was transferred to a 100 mL volumetric flask. The content was mixed with methanol (60 mL), sonicated for 20 min. to dissolve the drug as completely as possible. The solution was then filtered through a Whatman filter paper no. 41. The volume was adjusted up to the mark with methanol. An aliquot of this solution (3 mL) was transferred in to a 10 mL volumetric flask and Addition of 1 ml AML standard solution (100µg/ml) to the same volumetric flask. Methanol was transferred to this volumetric flask and Volume was made up to the mark to give a solution containing 90 µg/ml ALK, 13 µg/ml AML and 7.5 µg/ml HTZ . This solution was used for the estimation of ALK and AML.

Determination of the analytical wavelengths

Absorbance spectrum of pure ALK, AML and HTZ were scanned in the spectrum basic mode. By dilution of three standard drug solutions with methanol, solutions containing 80 µg/mL of ALK, 10 µg/ml of AML and 10 µg/ml of HTZ were scanned separately in the range of 200- 400 nm to determine the wavelength of maximum absorption for both all the drugs. ALK, AML and HTZ showed absorbance maxima at 279 nm, 360 nm and 271 nm respectively. The overlain spectra showed λmax of all drugs (Fig. 4). The arrangement of spectra of studied compound of favour is that AML has absorbance maxima at 360 nm and at that wavelength, in the studied concentration range; the spectra of ALK and HTZ solution show no absorbance. So the concentration of AML was calculated from the absorbance measured at maxima at 360 nm. Then, after calculating the AML concentration in investigated sample, the absorbance was established in which AML is participating at the measured wavelength for ALK and HTZ, i.e. at 279 nm and 271 nm. The concentration of ALK and HTZ in mixtures was calculated according to simultaneous equation method after eliminating the absorbance of AML at this wavelength.

Method validation[29]:

Calibration curve (Linearity)

Calibration curves were plotted over a concentration range of 4-28 µg/mL for AML, HTZ and 20-120 µg/mL for ALK. Accurately measured standard working solutions of AML, HTZ (0.4, 1.0, 1.6, 2.0, 2.4, and 2.8 mL) and ALK (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mL) were transferred to a three series

of 10 mL of volumetric flasks and diluted to the mark with methanol and absorbance were measured at 360 nm, 271 nm and 279 nm for three drugs. The calibration curves were constructed by plotting absorbance at 360 nm versus concentrations for AML and absorbance at 279 nm and 271 nm versus concentration for ALK and HTZ respectively.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of ALK, AML and HTZ by the standard addition method. Known amounts of standard solutions of AML (7, 10.5, 14 µg/mL), HTZ. (7, 10.5, 14 µg/mL) and ALK (20, 40, 60 µg/mL) were added to prequantified sample solutions of AML (10.5 µg/mL), HTZ (10.5 µg/mL) and ALK (40 µg/mL) tablet dosage form. The amounts of ALK, AML and HTZ were estimated by applying obtained values (n=6) to the regression equation of the calibration curve.

Method Precision (% Repeatability)

The precision of the instruments was checked by repeated scanning and measurement of absorbance of solution of (n = 6) of ALK (40 µg/mL), AML (20 µg/mL) and HTZ (20 µg/mL) without changing the parameter.

Intermediate Precision (Reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of ALK (40, 80, and 120 µg/mL), AML (10, 20, and 28 µg/mL) and HTZ (10, 20, and 28 µg/mL). The results were reported in terms of relative standard deviation (% RSD).

Limit of Detection and Limit of Quantification

LOD and LOQ of the drug were calculated using the following equations designated by International Conference on Harmonization (ICH) guideline:

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where σ = the standard deviation of the response
S = Slope of calibration curve.

Analysis of ALK and AML in combined dosage form:-

Pharmaceutical formulation of ALK, AML and HTZ was purchased from local pharmacy. The responses of formulations were measured at 279 nm, 271 nm and 360 nm for quantification of ALK, AML and HTZ as described above. These values were then equated in equation 1, 2 and 3 mentioned in section result and discussion and the concentration of each drug was calculated.

RESULTS AND DISCUSSION:

Method development

An attempt has been made to develop a fast, sensitive, precise, reproducible and economical analytical method for simultaneous estimation of ALK, AML and HTZ in their combined dosage form. In this method, the absorbance was measured at three wavelengths, one at 360 nm at that wavelength, no absorbance of ALK and HTZ solution. So the concentration of Amlodipine was calculated from the absorbance measured at maxima at 360 nm. Then, measured the absorbance at 279 nm and 271 nm. The concentration of ALK and HTZ in mixtures was calculated according to simultaneous equation method after eliminating the absorbance of AML at this wavelength. For this measurement, the solutions of ALK, AML and HTZ were prepared separately in methanol. They were scanned in the wavelength range of 200-400 nm. Data were recorded at an interval of 0.5 nm. From the overlain spectra of the three drugs (Fig 4) absorbencies were measured at selected wavelength. The absorbance of sample as above mention wavelength, substituted in the following equations (1, 2, and 3) to obtain the concentration ALK, HTZ and AML in g/100 ml respectively.

$$C_x = \frac{(A1*560) - (A2*350)}{11900} \quad (1)$$

$$C_y = \frac{(A1*30) - (A2*40)}{11900} \quad (2)$$

$$Cz = \frac{A3 + 0.009}{0.01} \quad (3)$$

Where,

C_X, C_Y and Cz = the concentration of ALK, HTZ and AML respectively.

A1 = the absorbance of mixture at 279 nm
A2 = the absorbance of mixture at 271 nm
A3 = the absorbance of mixture at 360 nm

Table 1 Regression analysis data and summary of validation parameters for the proposed method.

Parameters	Spectrophotometric Method		
	ALK at 279	AML at 360	HTZ at 271
Concentration range (µg/mL)	20-120	4-28	4-28
Slope	0.004	0.01	0.012
Intercept	0.001	0.009	0.076
Correlation coefficient	0.996	0.997	0.997
LOD (µg/mL)	2.41	0.67	0.289
LOQ (µg/mL)	7.32	2.03	0.87
% recovery (Accuracy, n = 6)	99.97 ± 0.82	99.93 ± 0.88	100.14 ± 0.81
Repeatability (% RSD, n = 6)	1.02	1.14	0.98
Precision (%RSD)			
Interday (n = 6)	0.74-1.92	0.91-1.73	0.20-1.44
Intraday (n = 6)	0.35-1.03	0.74-1.31	0.18-1.12

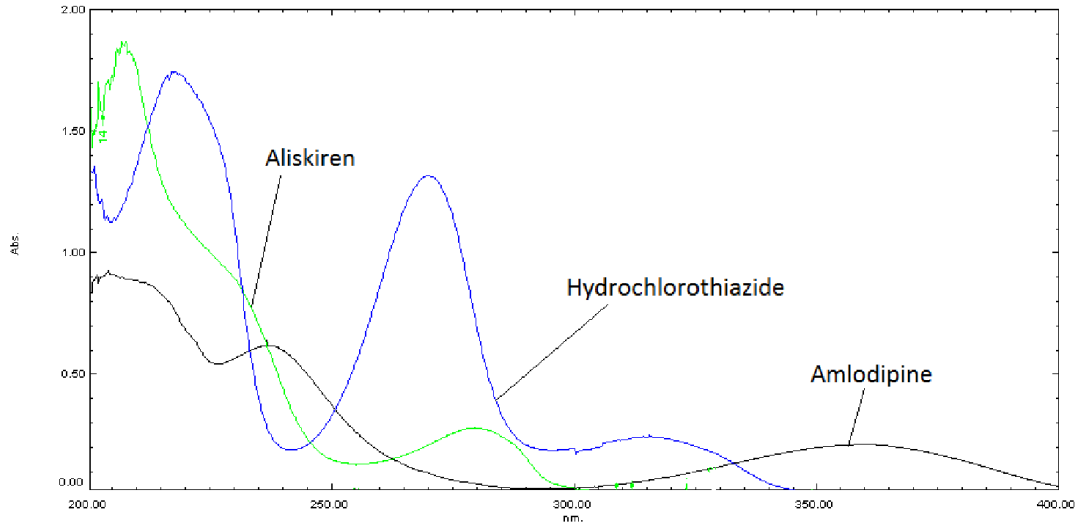

FIGURE: 4 Overlain absorption spectra of standard solution of Aliskiren,

Table 2 Recovery Data for the Proposed Method

Drug	Level	Amount of sample taken (µg/mL)	Amount of standard spiked (%)	Mean% Recovery ± SD*
ALK	I	40	50 %	98.58 ± 0.35
	II	40	100 %	101.2 ± 1.09
	III	40	150 %	100.13 ± 1.02
AML	I	10.5	50 %	99.42 ± 1.01
	II	10.5	100 %	101.5 ± 1.19
	III	10.5	150 %	98.87 ± 0.45
HTZ	I	10.5	50 %	99.12 ± 0.98
	II	10.5	100 %	101.23 ±0.35
	III	10.5	150 %	100.09 ±1.11

***** Mean % Recovery ± SD of six observations.

Amlodipine and hydrochlorothiazide in methanol

Validation of proposed method

The proposed method has been validated for the simultaneous determination of ALK, AML and HTZ in tablet dosage form using following parameters.

Linearity

Linear correlation was obtained between absorbance Vs concentrations of ALK in concentration range 20-120 µg/mL and in concentration range of 4-28 µg/mL for AML and HTZ. Regression parameters are mentioned in Table 1.

Accuracy

The recovery experiment was performed by the standard addition method. The mean recoveries were 99.97 ± 0.82, 99.93 ± 0.88 and 100.14 ± 0.81 % for ALK, AML and HTZ, respectively (Table 1). The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 2.

Method precision (% Repeatability)

The % RSD values for ALK, AML and HTZ were found to be 1.02, 1.14 and 0.98 for propose method (Table 1). The low values of RSD indicate the proposed methods are repeatable.

Intermediate precision

The low RSD values of interday (0.74-1.92%, 0.91-1.73 and0. 0.20-1.44%) and intraday (0.35-1.03, 0.74-1.31 and 0.18-1.12 %) variations for ALK, AML and HTZ, respectively, reveal that the proposed method is precise (Table 1).

LOD and LOQ

LOD for ALK, AML and HTZ were found to be 2.41 µg/mL, 0.67 µg/mL and 0.28 µg/mL, respectively. LOQ for ALK, AML and HTZ were found to be 7.32 µg/mL, 2.03 µg/mL and 0.87 µg/mL, respectively (Table 1). These data show that method is sensitive for the determination of ALK, AML and HTZ.

Assay of the pharmaceutical formulation

The proposed validated method was successfully applied to determine ALK, AML and HTZ in their combined dosage form. The spectrum of sample is shown in Fig. 4.4. The results obtained for ALK, AML and HTZ were comparable with the corresponding labeled amounts (Table 3).

The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the ALK, AML and HTZ in combined dosage form without any interference of the excipients.

Table 3 Analysis of Marketed Formulation of Aliskiren, Amlodipine and hydrochlorothiazide by Proposed Method (n = 6)

Sample No.	Label Claim			Amount Found			% Label Claim		
	ALK mg/tab	AML mg/tab	HTZ mg/tab	ALK mg/tab	AML mg/tab	HTZ mg/tab	ALK mg/tab	AML mg/tab	HTZ mg/tab
1	150	5	12.5	150.19	5.00	12.47	100.13	100.17	99.78
2	150	5	12.5	151.78	4.96	12.34	101.19	99.29	98.75
3	150	5	12.5	148.77	5.05	12.57	99.18	101.16	100.56
4	150	5	12.5	148.41	5.02	12.55	98.94	100.44	100.47
5	150	5	12.5	151.75	4.97	12.45	101.17	99.43	99.63
6	150	5	12.5	148.71	5.06	12.65	99.14	101.24	101.23
Mean				149.93	5.01	12.51	99.95	100.28	100.07
S.D.				1.55	0.04	0.11	1.03	0.83	0.87

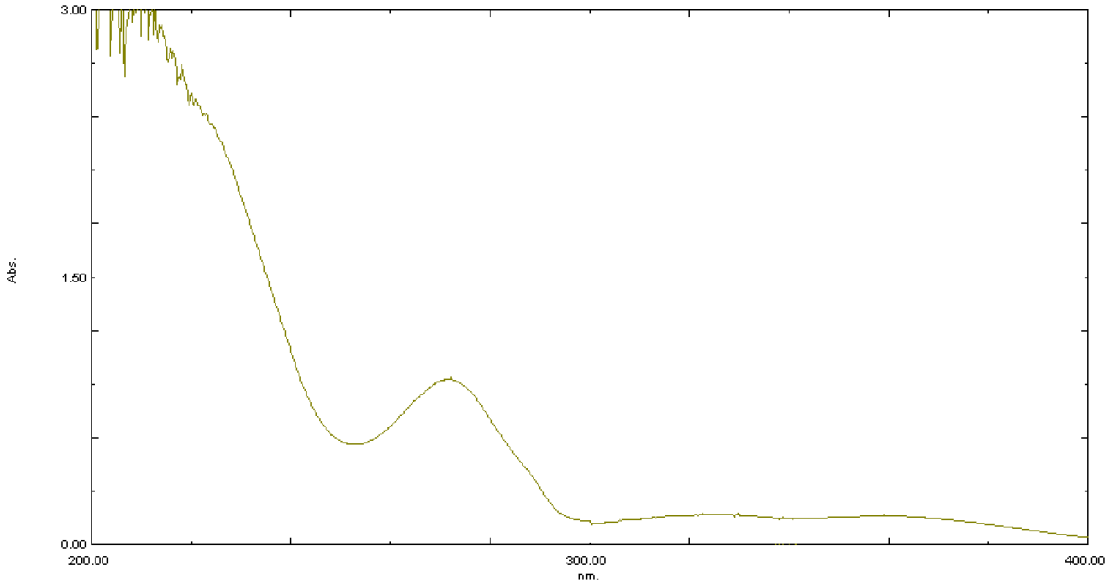

Figure 5 Absorption Spectra of sample solution of Aliskiren, Amlodipine and hydrochlorothiazide in methanol

CONCLUSION:

In this proposed method the linearity is observed in the concentration range of 20 – 120 µg/mL with co-efficient of correlation, (r^2) = 0.996 for ALK and 4 – 28 µg/mL with co-efficient of correlation (r^2) = 0.997 and 0.997 for AML and HTZ, respectively.

The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the ALK, AML and HTZ in combined dosage form without any interference of the excipients.

ACKNOWLEDGEMENTS:

The authors are greatly thankful to Atmiya Institute of Pharmacy, Yoghidham Gurukul for providing all the facilities to carry out the work.

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