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DEVELOPMENT AND VALIDTION OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF AZILSARTAN KAMEDOXOMIL AND AMLODIPINE BESYLATE IN SYNTHETIC MIXTURE

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ABSTRACT

A simple, economical, precise and accurate UV Spectroscopic method for simultaneous determination of azilsartan and amlodipine in synthetic mixture has been developed. First order derivative spectroscopy method (Method A) and absorbance ratio (Q-Absorbance) method (Method B) were used. The amplitudes at 227.50 nm and 247.76 nm in the first order derivative spectra were selected to determine azilsartan and amlodipine respectively. Amlodipine and azilsartan show an isoabsorptive point at 230.7 nm. The second wavelength used is 248.4 nm, which is the λ-max of azilsartan. Beer's law is obeyed in the concentration ranges of 4-32 µg/ml for both drug for derivative method as well as absorbance ratio method. The % assay in synthetic mixture was found to be in the range 99.50-100.5% for azilsartan and 98.40-99.41% for amlodipine by the proposed methods. Recovery was found in the range of 98.81-99.27% for azilsartan and 98.33-100.3% for amlodipine by first order derivative spectroscopic method and 98.33–99.83% for azilsartan and 98.68-100% for amlodipine by Q-absorbance ratio method. The results of analysis have been validated statistically and recovery studies confirmed the accuracy and reproducibility of the proposed methods which were carried out according to ICH guidelines.

KEYWORDS: Azilsartan, amlodipine, first order derivative spectroscopy, Q- absorbance, validation.

INTRODUCTION

Azilsartan kamedoxomil is chemically (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 2- ethoxy-1-([2'-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]methyl)-1H-benzimidazole-7-carboxylic acid, potassium salt. It is angiotensin II receptor antagonist. It stops vasoconstriction and decrease the blood pressure by blocking the angiotensin receptor via vasopressor hormone. Azilsartan is partially soluble in water and freely soluble in methanol and dimethyl sulfoxide (DMSO).

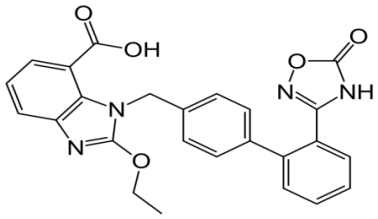

Figure 1: Chemical structure of azilsartan.

Amlodipine Besylate is chemically 3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. It is a synthetic long acting dihydropyridine calcium channel blocking agent. It has antihypertensive and antianginal properties and used in management of coronary artery disease. It lowers blood pressure by inhibiting the influx of calcium in cell. Amlodipine is used with or without other medications to treat high blood pressure. It is slightly soluble in water and freely soluble in methanol.

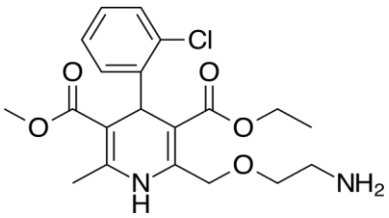

Figure 2: Chemical structure of amlodipine.

MATERIALS AND METHODS

Instrumentation

A Shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was

used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India).

Reagents and chemicals

Spectroscopic grade methanol was purchased from (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India). API of azilsartan and amlodipine was kindly supplied as a gift sample by Zydus Cadila Healthcare Ltd. Ahmedabad, India.

Preparation of Standard Stock Solutions and Calibration Curve

Standard stock solution of pure drug containing 100 µg/ml of azilsartan and 100 µg/ml of amlodipine were prepared separately in the methanol. These stock solutions were used to prepare series of solutions with conc. 4-32 µg/ml of azilsartan and amlodipine respectively for method A and method B and were used to prepare calibration curve.

Method A: First Order Derivative Spectroscopy

To determine derivative amplitude for azilsartan and amlodipine solution of increasing concentrations of azilsartan and amlodipine were prepared in combination and scanned in UV spectrum in the range 200 - 400 nm. These spectrums were converted to first order derivative spectra by using instrument mode is UV Probe 2.0. After observing the derivative amplitude of first order derivative spectra, it was observed that the first derivative spectra of azilsartan and amlodipine showed zero crossing points (Figure: 4). The first derivative spectra is adequate for determining azilsartan in the presence of amlodipine and vice versa. Azilsartan was determined by measurement of its amplitude at the zero crossing point of amlodipine at 227.50 nm, another side amlodipine was determined by measurement of it is at zero crossing point of azilsartan at 247.76 nm.

Method B: Absorbance Ratio (Q – Absorbance) Method

Absorbance ratio method uses the ratio of absorbencies at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that Azilsartan and Amlodipine show an isoabsorptive point at 230.80 nm (A1). The second wavelength used is 248.40 nm (A2), which is the λ-max of azilsartan. Five working standard solutions having concentration 4-32 µg/ml for azilsartan and amlodipine were prepared in methanol and the absorbencies at

230.80 nm (isoabsorptive point) and 248.40 nm (λ-max of azilsartan) were measured and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations.

CX = [(QM – QY) / (QX -QY)] × A1/ax1..... (1)

CY = [(QM – QX) / (QY -QX)] × A1/ay1 …… (2)

Where, A1 and A2 are absorbencies of mixture at 248.40 nm and 230.80 nm.

ax1 and ay1 are absorptivities of Azilsartan and Amlodipine 230.80 nm.

ax2 and ay2 are absorptivities of Azilsartan and Amlodipine respectively at 248.40 nm.

Where,

QM = A2 / A1, QX = ax2 / ax1 and QY = ay2 / ay1.

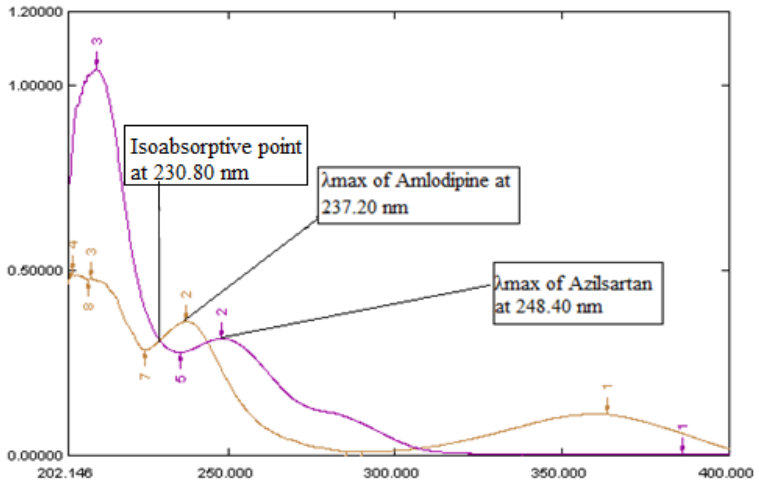

Figure 3: Overlay Spectra of Azilsartan 10 µg/ml and Amlodipine10 µg/ml.

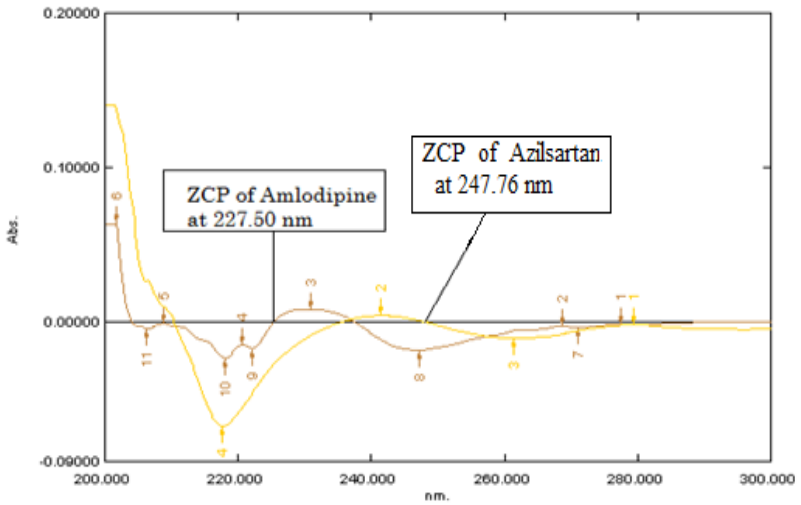

Figure 4: Overlay Derivative Spectra of Azilsartan 10 µg/ml and Amlodipine10 µg/ml.

Preparation of Sample Stock Solution and Synthetic Mixture analysis

A quantity of powder was taken from synthetic mixture equivalent to 20 mg of azilsartan (5 mg of amlodipine) was weighed and transferred to 100 ml flask containing 50 ml of methanol and ultrasonicated for 30 min, Filter the solution and transferred to 100 ml volumetric flask and volume was made up to mark by methanol. The solution was suitably diluted with methanol to have 200 µg/ml of azilsartan (50 µg/ml of Amlodipine) for method A and method B, respectively and samples were analyzed by the proposed methods.

Precision

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for azilsartan and amlodipine (24µg/ml and 6 µg/ml for drugs) without changing the parameter of the proposed spectrophotometry method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 Mixture of different concentrations of standard solutions of azilsartan and amlodipine (20+5, 24+6, 28+7 µg/ml for drugs).The result was reported in terms of relative standard deviation (% RSD).

Recovery studies

The accuracy of the method was determined by calculating the recoveries of azilsartan and amlodipine by the standard addition method. Known amounts of standard solutions of azilsartan and amlodipine were at added at 80, 100 and 120% level to prequantified sample solutions within the range of linearity for both the drugs.

Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$ Where, σ = the standard deviation of the response and S = slope of the calibration curve.

RESULT AND DISCUSSSION

In the present work, two methods namely, first derivative and Q-absorption ratio method, were developed for the simultaneous spectrophotometric estimation of azilsartan and amlodipine in synthetic mixture. Both drug were found to obey Beer's law in the concentration range of 4-32 µg/ml for methods. The validation parameters of azilsartan and amlodipine with respect to two methods are presented in table:I and table:II. The accuracy of the proposed methods were determined by recovery studies which presented in table:III, The percentages recovered were found to be in the range of 98-100.3, which showed that the excipients in synthetic mixture did not interfere with the analysis. The percentage of the individual drugs in the synthetic mixture according to the two methods were calculated and presented in the table: IV.

Table I: Results of validation parameters for first order derivative method.

Parameter	Azilsartan	Amlodipine
Beer's law range(µg/ml)	4-32	4-32
Wavelength(nm)	227.50	247.76
Correlation Coefficient	0.999	0.998
Slope	0.0017	0.0014
Intercept	0.0023	0.011
LOD (µg/ml)	1.21	1.31
LOQ(µg/ml)	3.67	3.88
Repeatability(%RSD, n=6)	0.9897	0.9082
Intraday Precision(%RSD, n=3)	0.614-0.994	0.513-0.804
Interday Precision(%RSD, n=3)	0.870-0.906	0.614-0.970
% Recovery	98.81-99.27	98.33-100.3

Table II: Results of validation parameters for absorbance ratio method.

Parameter	Azilsartan	Amlodipine	Isoabsorptive Point
Beer's law range (µg/ml)	4-32	4-32	4-32
Wavelength (nm)	248.40	237.20	230.80
Correlation Coefficient	0.999	0.998	0.998
Slope	0.0308	0.0293	0.0273
Intercept	0.0096	0.0591	0.0480
LOD (µg/ml)	0.332	0.926	1.237
LOQ (µg/ml)	1.007	2.809	3.75
Repeatability (%RSD, n=6)	0.875	0.79	0.924
Intraday Precision (%RSD, n=3)	0.523-0.737	0.607-0.842	0.672-0.874
Interday Precision (%RSD, n=3)	0.843-0.984	0.672-0.874	0.686-0.973
% Recovery	98.33-99.83	98.68-100.0	-

Table III: Results of recovery studies of azilsartan and amlodipine.

Method	Assay level (%)	Amount taken(mg)		Amount Added (mg)		Amount Recovered (mg)		% Recovery of standard added	
		AZL	AML	AZL	AML	AZL	AML	AZL	AML
A	0	12	3	-	-	11.94	2.95	99.50	98.10
	80	12	3	9.6	2.4	9.53	2.36	99.27	98.33
	100	12	3	12	3	11.88	3.02	99.00	100.3
	120	12	3	14.4	3.6	14.23	3.59	98.81	99.72
B	0	12	3	-	-	12.2	2.94	101.6	98.00
	80	12	3	9.6	2.4	9.44	2.39	98.33	99.58
	100	12	3	12	3	24.18	5.92	99.83	100.0
	120	12	3	14.4	3.6	26.57	6.48	99.79	98.68

Table IV: Results of simultaneous estimation in synthetic mixture for method A & B.

Method	Label Claim (mg)		Amount Found(mg)		% Assay	
	AZL	AML	AZL	AML	AZL	AML
A	20	5	20.30	4.97	101.5	99.41
B	20	5	19.90	4.92	99.50	98.40

CONCLUSION

The proposed spectrophotometric method was found to be are new, rapid, accurate, precise, sensitive and reproducible and can be employed for routine analysis for simultaneous estimation of azilsartan and amlodipine in synthetic mixture. Methanol was used as the solvent since both the drugs exhibit good solubility in it and no interference due to excipients of synthetic mixture were observed.

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