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Simultaneous spectrophotometric determination of Azilsartan Kamedoxomil and Cilnidipine in mixture

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Abstract
The objective of the current study was to develop a simple, sensitive, accurate and precise dual wavelength spectrophotometric method for simultaneous determination of Azilsartan Kamedoxomil and Cilnidipine in combined dosage form. The principle for dual wavelength method is the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest. The wavelengths selected for determination of Azilsartan Kamedoxomil are 229.4 nm and 256.9 nm at which Cilnidipine showed same absorbance, whereas the wavelengths selected for determination of Cilnidipine are 238.1 nm and 258.8 nm at which Azilsartan Kamedoxomil showed same absorbance. Methanol is used as a solvent. Regression analysis of Beer's plots showed good correlation in concentration range of 2-30 µg/ml for Azilsartan Kamedoxomil and 2-25 µg/ml for Cilnidipine. The method was validated according to ICH guidelines for various parameters like linearity, precision (method precision and intermediate precision), accuracy (recovery study), LOD and LOQ. The proposed method is successfully applied for simultaneous estimation of both drugs from synthetic mixture. The assay and recovery results are found satisfactory; hence the method can be applied for the routine QC analysis of both drugs in combination.

Keywords: Dual wavelength method, azilsartan kamedoxomil, cilnidipine, validation, synthetic mixture, recovery

Introduction

Azilsartan Kamedoxomil (AZL) (Figure 1) is an Angiotensin II receptor antagonist, which 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 - carboxylate monopotassium salt [1]. It is a white crystalline powder which is practically insoluble in water, freely soluble in methanol, dimethyl sulfoxide and dimethylformamide. AZL has an ability to remain tightly bound to the AT1 receptor for a very long period of time. It lowers blood pressure by inhibiting the action of angiotensin II, a vasopressor hormone. AZL is used for the treatment of essential hypertension [1, 2].

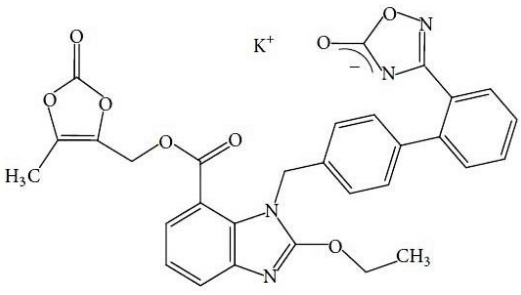

Fig 1: Chemical structure of Azilsartan Kamedoxomil

Cilnidipine (CIL) (Figure 2) is a novel and unique dihydropyridine calcium channel blocker, which is chemically 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine carboxylic acid-2-methoxyethyl-(2E)-3-phenyl-propenyl ester [3]. It is a light yellowish powder. CIL is a dual blocker of L-type voltage-gated calcium channels in vascular smooth muscles and N-type calcium channels in sympathetic nerve terminals that supply blood vessels. It possesses a slow-onset, long-lasting vasodilating effect [2-4].

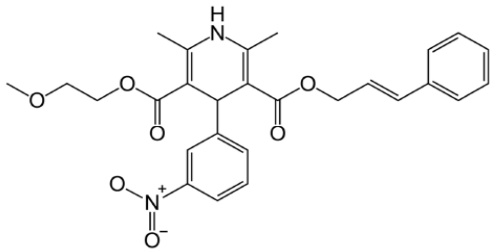

Fig 2: Chemical structure of Cilnidipine

AZL, CIL and their combination are not official in any pharmacopeia. So, there is no official method for their estimation alone and in combination. Extensive literature survey reveals that various analytical methods like Spectrophotometric [6-8], Spectrofluorimetric [8-9], HPTLC [10-11], HPLC [12-17], LC-MS [18], UPLC MS/MS [19] are reported for analysis of AZL alone as well as in combination with other drugs. Literature survey also reveals spectrophotometric [20-21], HPTLC [22-23], HPLC [24-32], UPLC [33], LC-MS/MS [34] methods for analysis of CIL alone as well as in combination with other drugs. Literature survey does not reveal any

analytical method for simultaneous estimation of AZL and CIL in synthetic mixture or dosage forms. So, an attempt has been made to develop an accurate, precise and economically viable spectrophotometric method for simultaneous estimation of both drugs from synthetic mixture.

Materials and Method

Materials

Instruments and Apparatus

The instrument used was a double beam UV-visible spectrophotometer (Shimadzu, UV-1700, Japan) attached to UV-probe 2.0 system software, with a spectral width of 2 nm, wavelength accuracy of ±0.5nm and a pair of 1 cm matched quartz cells. All weighing was done on an electronic analytical balance (CP224S, Sartorius, Germany). An ultrasonic cleaner (Frontline FS 4, Mumbai, India) was also used. Calibrated glass wares were used throughout the work.

Reagents

Pure drug powders of AZL and CIL were obtained as gift samples from Zydus Cadila Healthcare Ltd., Ahmedabad, India. Methanol of AR Grade was supplied by S.D. Fine Chemicals, Mumbai, India. Whatman filter paper no 41 (Millipore, USA) was also used.

Method

Preparation of Solutions

Preparation of standard stock solutions

Accurately weighed quantity of AZL (10 mg) and CIL (10 mg) was transferred to two separate 100 ml volumetric flasks and dissolved in little amount of methanol. Then the flasks were shaken for few minutes and made up to the mark with methanol to obtain standard stock solution having concentration, 100 µg/ml for both drugs.

Preparation of working standard solutions

The working standard solutions were prepared by transferring appropriate aliquots of standard stock solution of AZL (0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 3.0 ml) and CIL (0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.5 ml) into a series of 10 ml volumetric flasks. The volume of solutions was adjusted to the mark with methanol to get the concentration in a range of 2-30 µg/ml for AZL and 2-25 for CIL.

Preparation of synthetic mixture

The synthetic mixture was prepared by mixing AZL (400 mg) and CIL (100 mg) with excipients (300 mg) like starch, magnesium stearate, lactose and talc.

Method Development

Selection of analytical wavelengths

In this dual wavelength method for estimation of both analyte, two wavelengths have been selected at which one analyte shows same absorbance and at these two wavelengths, difference in absorbance is used for estimation of second analyte. Standard solutions of AZL and CIL were prepared in methanol and scanned in the UV range of 200-400 nm. From overlain spectra, two wavelengths 229.4 and 256.9 nm (λ_1 and λ_2) were selected on spectra at which AZL showed some absorbance difference while difference is zero for CIL. Similarly, two wavelengths 238.1 nm and 258.8 nm (λ_3 and λ_4) were selected on spectra at which CIL showed some absorbance difference while difference is zero for AZL. These four selected wavelengths were employed for the estimation of both the drugs, respectively.

Method Validation

The developed method was validated as per ICH Q2 (R1) guidelines in terms of linearity, precision, accuracy and limit of detection and quantitation [35].

Linearity

For calibration curve, accurately measured aliquots of standard stock solution of AZL (0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 3.0 ml) and CIL (0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.5 ml) were transferred into a series of 10 ml volumetric flasks and diluted up to the mark with methanol to obtain a concentration range of 2-30 µg/ml for AZL and 2-25 µg/ml CIL. The absorbances of resulting solutions were measured at selected wavelengths for AZL and CIL against methanol as a blank. After that, absorbance difference was plotted against concentration and regression line equations were obtained.

Precision

The precision of the method was determined in terms of i) Method precision and ii) Intermediate precision:

i) Method Precision (Repeatability

In method precision, the concentration of AZL (12 µg/ml) and CIL (3 µg/ml) was repeated for six times. The absorbances were measured on the same day without changing the parameters of a developed method. The results were reported in terms of % Relative Standard Deviation (RSD).

ii) Intermediate precision (Intraday and Interday Precision)

In intermediate precision, the absorbances of standard solutions of AZL and CIL (4, 8 and 12 µg/ml) were measured three times on the same day (intraday precision) and on three different days (interday precision) over the period of one week. The results were reported in terms of % RSD.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The sensitivity of method is described in terms of LOD and LOQ. The values of LOD and LOQ for AZL and CIL were measured by using the following equation designated by ICH guidelines:

$$LOD = 3.3 \times \sigma/S$$
$$LOQ = 10 \times \sigma/S$$

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

Accuracy

To demonstrate the accuracy of the proposed method, recovery study was carried out through standard addition method by adding a known amount of the standard solutions of AZL and CIL at three different levels (80%, 100% and

120%) to the pre-quantified sample solution. Percentage recovery was then calculated. The experiment was conducted thrice.

Assay of Synthetic Mixture

The synthetic mixture amount equivalent to 40 mg AZL and 10 mg CIL was accurately weighed and transferred into 100 ml volumetric flask. Abousssssssst 40 ml methanol was added to the flask and sonicated for 12-15 min. The final volume was adjusted with methanol and filtered through Whatman filter paper No.41. Then, the resulting solution was suitably diluted to get final sample solution containing AZL (12 µg/ml) and CIL (3 µg/ml). After that, the absorbance of sample solution was measured at selected wavelengths for AZL and CIL. Lastly, the concentration of AZL and CIL present in the sample solution was obtained by putting respective response into the regression equation of AZL and CIL for the proposed method.

Results and Discussion

Method Development

The spectra of the drugs suggested that a dual wavelength spectrophotometric method was a suitable method for simultaneous determination of AZL and CIL. Methanol was taken as solvent system. Wavelengths 229.4 nm and 256.9 nm (difference is zero for CIL) were selected for determination AZL, whereas 238.1 nm and 258.8 nm (difference is zero for AZL) were selected for determination of CIL. The Overlain spectra of AZL (10 µg/ml) and CIL (10 µg/ml) is shown in Figure 3.

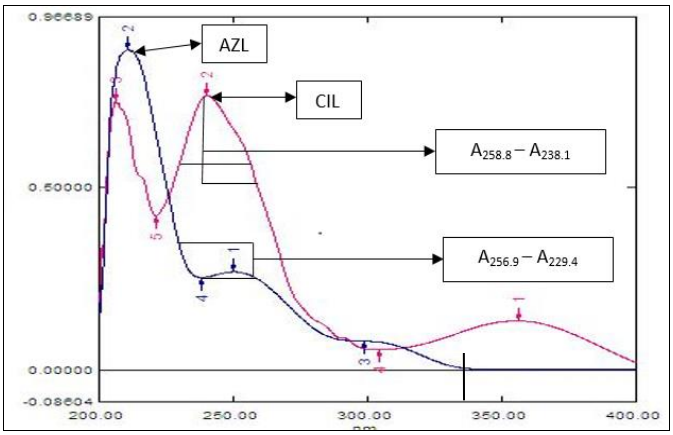

Fig 3: Overlain spectra of AZL (10 µg/ml) and CIL (10 µg/ml) in methanol

Method Validation

Validation parameters were studied at all selected analytical wavelengths as per ICH guidelines. The calibration curves of AZL and CIL were linear in the concentration range of 2-30 µg/ml and 2-25 µg/ml, respectively. Characteristics parameters of regression equation and correlation are given in Table 3. Recovery study was achieved by standard addition method at three different concentration levels (80%, 100% and 120%). The result of recovery study was found to be satisfactory for both drugs and is shown in Table 1. The result of assay obtained by the proposed method reveals non-interference from the common excipients of synthetic mixture and is shown in Table 2. Results of other validation parameters including Repeatability, Intraday, Interday, LOD and LOQ were found to be within acceptance criteria and are shown in Table 3.

Table 1: Recovery data of AZL and CIL by dual wavelength method

Drug	Level	Amount Taken (µg/ml)	Amount Added (%)	% Mean Recovery ± S.D (n=3)
AZL	1	6	80	99.55 ± 0.49
	2	6	100	101.3 ± 0.31
	3	6	120	100.7 ± 0.71
CIL	1	1.5	80	99.15 ± 0.37
	2	1.5	100	100.8 ± 0.25
	3	1.5	120	101.0 ± 0.57

S.D = Standard Deviation and n = number of replicates.

Table 2: Assay Results of AZL and CIL in synthetic mixture by dual wavelength method

Sr. No.	Label Claim (mg)		Amount Found (mg)		% Label Claim ± S.D (n=6)	
	AZL	CIL	AZL	CIL	AZL	CIL
1	40	10	40.22	10.02	100.6 ± 0.65	100.2 ± 1.06

S.D = Standard Deviation and n = number of replicates.

Table 3: Regression analysis data and summary of validation parameters for dual wavelength method

Parameters	AZL	CIL
Wavelength range (nm)	229.4 - 256.9	238.1 - 258.8
Beer's law limit (µg/ml)	2-30	2-25
Regression equation (y = mx + c)	y = 0.0174x + 0.0195	y = 0.0101x - 0.0029
Slope (m)	0.0174	0.0101
Intercept (c)	0.0195	0.0029
Correlation Coefficient (r^2)	0.9998	0.9996
Repeatability (n=6) (%RSD)	0.61	0.90
Intraday (n=3) (%RSD)	0.46 - 0.73	0.27 - 0.59
Interday (n=3) (%RSD)	0.92 – 1.03	0.54 – 0.75
LOD (µg/ml)	0.34	0.29
LOQ (µg/ml)	1.03	0.89
Accuracy (n=3) (% Recovery ± S.D)	100.5 ± 0.50	100.3 ± 0.40
% Assay ± S.D (n=6)	100.6 ± 0.65	100.2 ± 1.06

RSD = Relative Standard Deviation, LOD = Limit of Detection, LOQ = Limit of Quantification, S.D = Standard Deviation and n = number of replicates.

Conclusion

The proposed dual wavelength method gives accurate and precise results for determination of AZL and CIL in synthetic mixture. There is no interference in the analysis from additives present in the synthetic mixture; hence the method can be easily used for routine analysis. The most striking features of the dual wavelength method are its simplicity and rapidity. Method validation has been demonstrated by variety of tests for linearity, accuracy, precision, LOD and LOQ, results are found to be within acceptance criteria of ICH guidelines.

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