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Research Article

Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Olmesartan Medoxomil and Cilnidipine by Simultaneous Equation Method

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ABSTRACT

UV Spectrophotometric method has been developed for simultaneous estimation of Olmesartan Medoxomil (OLME) and Cilnidipine (CILNI) in bulk drug and in laboratory mixture. This method utilizes methanol as a solvent and λmax of Olmesartan Medoxomil and Cilnidipine selected for analysis was found to be 241 nm and 253 nm respectively. Linearity was observed in the Olmesartan Medoxomil concentration range of 4-20 µg/ml and Cilnidipine concentration range 2 -10 ug/ml (r^2 = 0.998 and r^2 0.999) of both drugs. The accuracy and precision were determined and found to comply with ICH guidelines. This method showed good reproducibility and recovery with % RSD in the desired range. The proposed methods can be successfully applied for the routine analysis of both the drugs. This method was simple, rapid, accurate, and sensitive.

Keywords: Olmesartan Medoxomil, Cilnidipine, UV spectroscopy, Simultaneous Equation method

INTRODUCTION

Olmesartan Medoxomil (OLME) (fig. 1a) is the chemically known as, 2,3-dihydroxy-2-butenyl4(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-ylphenyl)benzyl]imidazole carboxylate, cyclic 2,3-carbonate. Olmesartan is a prodrug that works by blocking the binding of angiotensin II to the AT_1 receptors in vascular muscle; it is therefore independent of angiotensin II synthesis pathways, unlike ACE inhibitors. By blocking the binding rather than the synthesis of angiotensin II, olmesartan inhibits the negative regulatory feedback on renin secretion. As a result of this blockage, olmesartan reduces vasoconstriction and the secretion of aldosterone[1-3]. Cilnidipine(CILNE) (fig. 1b) the chemically known as, *o*3-(2-methoxyethyl) *O*5-[(*E*)-3-phenylprop-2-enyl]2,6-dimethyl-4-(3-nitrophenyl)-1,4dihydropyridine-3,5-dicarboxylate. Cilnidpiine is it reduces the incidense of pedal edema unlike amlodipine. Cilnidipine due to its blocking action at N-type calcium channel dilates both arteriole & venules as a result the pressure in the capillary bed is reduces. The accumulated fluid in the tissues flows back to veins & thus Cilnidipine minimizes the incidence of pedal edema[1-4]. Combination drug products of OLME and CILNI are widely marketed and used in the treatment of hypertension[5-7]. Several analytical methods like UV spectrophotometry, HPLC, HPTLC, UPLC have been reported for estimation of OLME & CILNI by single drug and also by combining with other drugs. However no method has been reported till date for the simultaneous estimation of OLME & CILNI using the UV spectrophotometric method. The present paper describes the development and validation of

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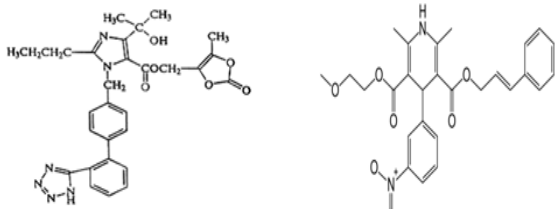
two analytical methods for simultaneous estimation of OLME & CILNI by UV spectrophotometry in tablet dosage form; the methods include simultaneous equation method & absorbance ratio method.[8-12] The proposed methods are optimized and validated as per the ICH guidelines [13-14].



(1a) (1b)

Fig. 1 Chemical structures of the analytes (1a) OLME & (1b) CILNI

MATERIALS AND METHODS

Instrumentation

Double beam UV-visible spectrophotometer (hελios Alpha, Model - V 7.09) having two matched quartz cells with 1 cm light path. An Electronic analytical balance (Contech, CA34 Model) was used in the study.

Material and reagent:

Double distilled water and Whatmann filter paper (0.45µm) were used for filtration. Active pharmaceutical ingredient (API) working standards of Olmesartan medoxomil (OLME), was obtained as gift sample from Intas Pharma limited, Ahmadabad, Indaa and Cilnidipine(CILNI) was obtained as gift sample from J. B. Chemicals, Surat, Gujarat, India and test samples (tablets with composition OLME-40 mg and CILNI – 10mg) were procured from the local market.

Preparation of Standard Stock solution of OLME and CILNI:

Accurately weighed quantity of Olmesartan medoxomil 25 mg was transferred to 25 ml volumetric flask, dissolved in 10 ml of methanol and diluted up to mark with methanol to give a stock solution having strength of 1mg/ml.

Preparation of Working Standard Solution of OLME and CILNI:

100 µg/ml of OLME and CILNI solution were prepared by diluting 10 ml of stock solution to 100 ml with methanol in separate 100 ml volumetric flask. Suitable aliquots of this solution were diluted up to the mark with methanol to get the concentration range of 7,9,11,13,15 µg/ml for OLME and 3,4,5,6,7 µg/ml for CILNI.

(A) Preparation of Working Standard Solution OLME

100 µg/ml of OLME solution was prepared by diluting 1 ml of stock solution with methanol in 10 ml volumetric flask up to the mark.

(B) Preparation of Standard Solution of Cilnidipine

Preparation of Standard Stock Solution of CILNI

Accurately weighed quantity of Cilnidipine 25 mg was transferred to 25 ml volumetric flask and make up to methanol and sonicate for 30 min for dissolving drug, to give a stock solution having strength of 1mg/ml.

Preparation of Working Standard Solution CILNI

100 µg/ml of CILNI solution was prepared by diluting 2.5 ml of stock solution with methanol in 25 ml volumetric flask up to the mark.

Procedure for Determination of Wavelength for Measurement

1.0 ml of working standard stock solution of OLME (100 µg/ml) and 1.0 ml of working standard stock solution of CILNI (100 µg/ml) were pipette out into two separate 10 ml volumetric flask and volume was adjusted to the mark with methanol to get 9µg/ml of OLME and 6 µg/ml of CILNI. Each solution was scanned

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between 200 - 400 nm against methanol as a reagent blank. Wavelengths were selected from the overlay spectra of OLME and CILNI.

Preparation of Calibration Curve

Calibration Curve for OLME

Calibration curve for OLME consists of different concentrations of standard OLME solution ranging from 4-20 µg/ml. The solutions were prepared by pipetting out 0.7, 0.9, 1.1, 1.3, and 1.5 ml of the working standard solution of OLME (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol. The absorbance of the solutions was measured at 253 nm and 241 nm against methanol as a reagent blank. Calibration curve was plotted at both wavelengths and two equations were formed using the specific absorbance (absorptivity).

(B) Calibration Curve for CILNI

Calibration curve for CILI consisted of different concentrations of standard CILNI solution ranging from 1-10 µg/ml. The solutions were prepared by pipetting out 0.3, 0.4, 0.5, 0.6 and 0.7 ml of the working standard solution of CILNI (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol.

The absorbance of the solutions was measured at 253 nm and 241 nm against methanol as a reagent blank. Calibration curve was plotted at both wavelengths and two equations were formed using the specific absorbance (absorptivity).

Validation of proposed method

Parameters to be considered for the validation of method are:

Linearity and Range

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 4 - 20 µg/ml and 1-10 µg/ml for OLME and CILNI respectively.

The calibration curve of absorbance vs. respective concentration was plotted and correlation coefficient and regression line equations for OLME and CILNI were calculated.

Precision

I) Repeatability

Aliquots of 1.1 ml of working standard solution of OLME (100 µg/ml) were transferred to 10 ml volumetric flask and Aliquots of 0.5 ml of working standard solution of CILNI (100 µg/ml) were respectively transferred to the same above 10 ml volumetric flask. The volume was adjusted up to mark to make 11 µg/ml of OLME and 5 µg/ml of CILNI solution was analysed 6 times on the same day spectrophotometry and % R.S.D. was calculated.

II) Intraday precision

Aliquots of 0.9, 1.1, and 1.3 ml of working standard solution of OLME (100 µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 0.4, 0.5 and 0.6 ml of working standard solution of CILNI (100 µg/ml) were respectively transferred to the same above series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 9, 11, and 13 µg/ml solution of OLME and 4, 5 and 6 µg/ml solution of CILNI. Solution was analysed 3 times on the same day spectrophotometry and % R.S.D. was calculated.

III) Interday Precision

Aliquots of 0.9, 1.1, and 1.3 ml of working standard solution of OLME (100 µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 0.4, 0.5 and 0.6 ml of working standard solution of CILNI(100 µg/ml) were respectively transferred to the same above series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 9.0, 11, and 13 µg/ml solution of OLME and 4, 5 and 6 µg/ml solution of CILNI. Solution was analyzed 3 times on the 3 different day using spectrophotometry and % R.S.D. was calculated.

Accuracy

The accuracy of the method was performed

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by conducting the recovery studies (80, 100 and 120%) of pure drugs from marketed formulation, by standard addition method. The actual and measured concentrations were then compared.

LOD (Limit of Detection)

The LOD is estimated from the set of 6 calibration curves used to determine method linearity.

The LOD may be calculated as,

LOD = 3.3 σ/S

where, σ = The standard deviation of Y-intercept of 6 calibration curves.

S = The mean slope of the 6 calibration curves.

LOQ (Limit of Quantification)

The LOQ is estimated from the set of 6 calibration curves used to determine method linearity.

The LOQ may be calculated as,

LOD = 10 σ/S

where,

σ = The standard deviation of Y- intercept of 6 calibration curves.

S = The mean slope of the 6 calibration curves.

Simultaneous Estimation of OLME And CILNI in Combined Dosage Form

The powder of twenty tablets were weighed. An accurately weighed quantity of the powder equivalent to about 40mg of OLME was taken in 10 ml volumetric flask and dissolved with methanol and further diluted upto the mark with same solvent. The solution was then filtered through the Whatman filter paper No. 41. Necessary dilutions are made with methanol to give final concentration 40 µg/ml and 10 µg/ml of Olmesartan Medoxomil and Cilnidipine respectively. The solutions are then scanned between 200-400nm and absorbances are measured at respective wavelengths. The concentration of each drug was calculated using equation of straight line.

Absorbance of the resulting solution was measured at 253 nm and 241 nm against methanol. The concentration of OLME and CILNI can be obtained as

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2}$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$

Where, A_1, A_2 are absorbance of mixture at 253 nm (λ_1) and 241 nm (λ_2) respectively,

a_{x1} and a_{x2} are absorptivity of OLME at λ_1 and λ_2 respectively,

a_{y1} and a_{y2} are absorptivity of CILNI at λ_1 and λ_2 respectively,

C_x and C_y are concentrations of OLME and CILNI respectively.

RESULTS AND DISCUSSION

Selection of Wavelength for Simultaneous Estimation of OLME and CILNI

To determine wavelength for measurement, standard spectra of OLME and CILNI were scanned between 200-400 nm against methanol. Absorbance maxima were obtained at 253 nm and at 241nm for OLME and CILNI respectively.

Method Validation:

The linearity range for OLME and CILNI were 7-15 µg/mL and 3-7 µg/mL respectively. Recovery studies was carried out by addition of standard drug solution to pre-analyzed dosage form solution at three different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug sample. The results of the recovery studies are found to be satisfactory for OLME and CILNI and shown in Table 1 and 2 respectively. The result of assay procedure obtained was showed in Table 3. Summary of Other validation parameters

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including Repeatability, Intraday, Interday, LOD and LOQ were found to be satisfactory and are shown in Table 5.

CONCLUSION

The results obtained by applying the suggested procedures, it is proved that the proposed method is accurate, precise, simple, sensitive, selective and rapid and can be applied successfully in routine analysis for the estimation of OLME and CILNI in their combined pharmaceutical dosage form. The developed method was validated as par ICH guidelines.

Figure-2: Overlay spectra of OLME and CILNI showing λ_{max} in Methanol

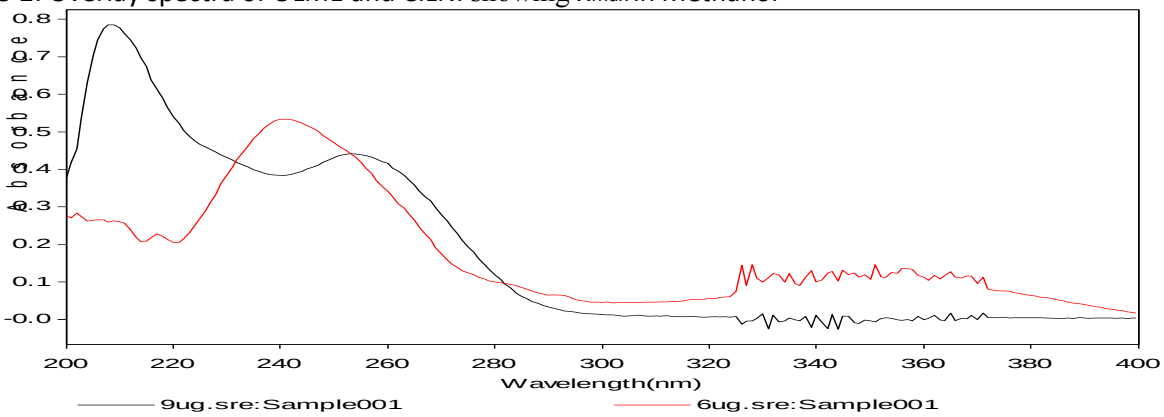

Figure-3: Overlay spectra of OLME in Methanol showing Linearity

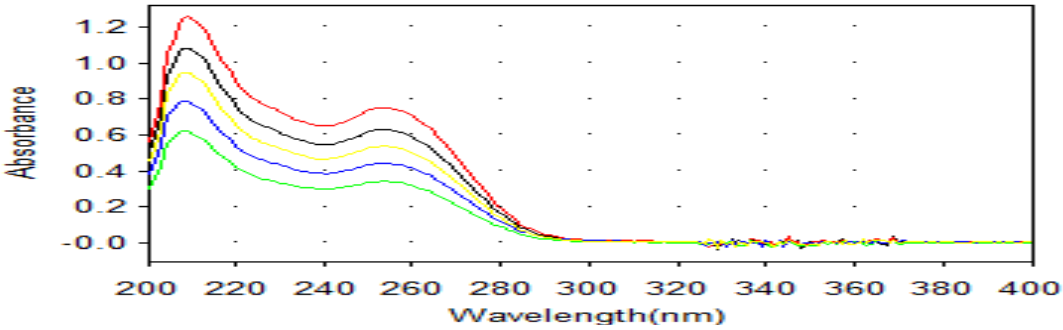

Figure-4: Overlay spectra of CILNI in Methanol showing Linearity

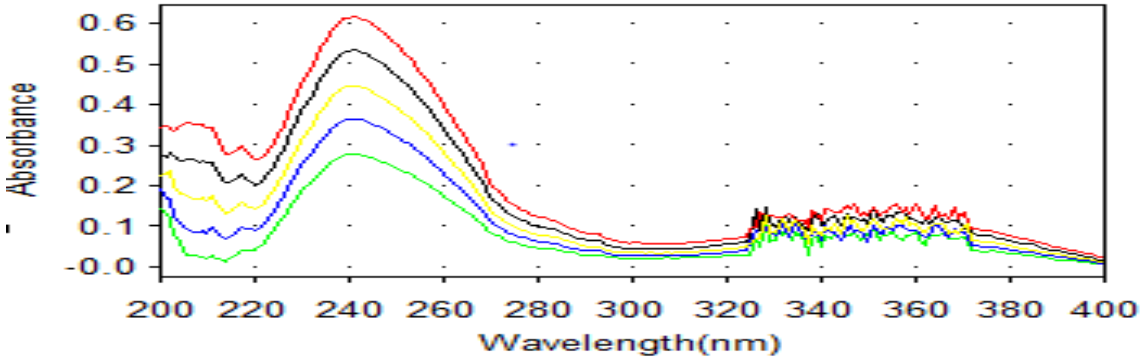

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Table-1 Result of Recovery Studies for OLME in dosage form:

Amount of OLME in mixture (µg/ml)	Amount of Std OLME added (µg/ml)	Total amount of OLME (µg/ml)	Total amount of OLME found (µg/ml) Mean* ± SD	%Recovery
11	8.8	19.8	19.37 ± 0.023	97.82
11	11	22	22.29 ± 0.001	101.31
11	13.2	24.2	24.31 ± 0.0020	100.45

[*=mean value of 3 determination]

Table-2 Result of Recovery Studies for CILNI in dosage form:

Amount of CILNI in Mixture (µg/ml)	Amount of Std CILNI added (µg/ml)	Total amount of CILNI (µg/ml)	Total amount of CILNI found (µg/ml) Mean* ± SD	%Recovery
5	4	9	9.15±0.0151	101.66
5	5	10	10.10±0.00057	101
5	6	11	11.27±0.003055	102.45

[*=mean value of 3 determination]

Table-3: Analysis of OLME and CILNI in dosage form:

Tablet dosage form	Label claim(mg)		%Recovery ± SD (% of label claim*)	
	OLME	CILNI	OLME	CILNI
	40 mg	10 mg	98.75 ± 0.6186	101 ± 0.54477

[*=mean value of 5 determination]

Table-4: Regression Characteristics:

Characteristics	OLME at 253 nm	OLME at 241 nm	CILNI at 253 nm	CILNI at 241 nm
Linearity (µg/ml)	4-20	4-20	1-10	1-10
Regression Equation	y = 0.050x - 0.014	y = 0.043x - 0.007	y = 0.071x + 0.015	y = 0.085x + 0.022
Slope	0.050	0.043	0.071	0.085
r2	0.998	0.997	0.999	0.999
Intercept	-0.014	0.007	0.015	0.022
S.D. of Intercept	0.005508	0.005565	0.005292	0.002517

TABLE-5: VALIDATION PARAMETERS:

Parameters	OLME at 253 nm	OLME at 241nm	CILNI at 253nm	CILNI at 241nm
Repeatability (%RSD) (n=6)	0.1289	0.1622	0.2656	0.1242
Precision (%RSD)				

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Intra-day (n=3)	0.3232-1.0129	0.2624-2.5147	0.2553-0.5998	0.2252-0.7112
Inter-day (n=3)	0.3183-0.5510	0.400-3.6870	0.4174-0.6715	0.8049-1.3249
LOD (µg/ml)	0.363	0.4226	0.245	0.097
LOQ (µg/ml)	1.102	1.293	0.745	0.295
% Recovery (n=3)	97.82%-101.31%			100.86%-102.45%
Assay (mean ± S.D.) (n=5)	98.75±0.6186			101 ±0.54497

LOD: Limit of Detection, LOQ: Limit of Quantitation, R.S.D.: Relative standard deviation, S.D.: Standard deviation

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