DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF CILNIDIPINE AND OLMESARTAN MEDOXIMIL IN TABLET DOSAGE FORM

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ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical spectrophotometric method based on simultaneous equation method for the simultaneous estimation of cilnidipine and olmesartan medoximil in combined tablet dosage form. The method is based on the simultaneous equation for analysis of both the drugs using methanol as solvent. Cilnidipine has absorbance maxima at 256 nm and olmesartan medoximil has absorbance maxima at 240 nm in methanol. The linearity was obtained in concentration range 2-20 µg/ml for both cilnidipine and olmesartan medoximil. The concentration of drugs was determined by using simultaneous equation method. The mean recovery was 99.87% - 100.29% and 99.86% - 100.13% for cilnidipine and olmesartan medoximil respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for simultaneous determination of cilnidipine and olmesartan medoximil in pharmaceutical dosage form. The result of analysis have been validated statistically and by recovery studies.

KEY WORDS

Validation, Simultaneous Equations, Cilnidipine, Olmesartan Medoximil.

INTRODUCTION

Cilnidipine O3-(2-methoxyethyl) O5-[(E)-3-phenylprop- 2- enyl] 2, 6- dimethyl- 4- (3-nitrophenyl)-1, 4-dihydropyridine- 3,5-dicarboxylate is a novel and unique dihydropyridine calcium channel blocker that possesses a slow onset, long lasting vasodilating effect. Cilnidipine shows first pass metabolism. [1] **Olmesartan medoximil** is a pro drug which after ingestion liberates the only active metabolite, olmesartan.[2] it is a competitive and selective all type 1 receptor antagonist that is used alone or with other antihypertensive agents to treat hypertension ,the hydrolysis of olmesartan medoximil occurs readily by the action of esterases,which are present abundantly in gastrointestinal tract, liver and plasma. olmesartan blocks the vasoconstrictor effects of Angiotensin II by selectivity blocking the binding of

Angiotensin II to the AT1 receptor in vascular smooth muscle for the treatment of hypertension [3] Literature survey reveals that various methods like Spectrophotometric, HPLC and HPTLC etc. have been reported for Cilnidipine and Olmesartan medoximil individual and combination with other drugs like Telmisartan, Metoprolo succinate etc. Based on literature, no method has been reported for simultaneous estimation of Cilnidipine and Olmesartan medoximil in their combined dosage form.

MATERIALS AND METHOD

Apparatus

A Shimadzu model THERMO Limited, model α double beam UV/Visible spectrophotometer with spectral width of 2 nm. Wavelength accuracy of 0.5 nm and

pair of 10 mm matched quartz cell was used to measure absorbance of all the solution. Spectra were automatically obtained by UV-Probe system software (UV Probe version 2.10).

REAGENTS AND MATERIALS

API **of** Cilnidipine was kindly gifted by Pure Chem. Lab Ankleswar. API of Olmesartan medoxomil was kindly gifted by Cadila pharma Ahmadabad. Olmesartan medoxomil and Cilnidipine combined dosage form (NEXOVAS-O) purchased from local market. All other reagents used were of analytical grade.

Preparation of standard stock Solution

An accurately weighed quantity of CIL 10 mg and OLM 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 concentration of CIL 100 µg/ml and OLM 100 µg/ml.

Method

The standard solution of CIL 10 µg/mL and OLM 10 µg/mL were scanned separately in the UV range of 200-400 nm to determine λmax of the both the drugs. The λmax of CIL and OLM were found to be 256 nm and 240 nm respectively. Five standard solution having concentration 2, 4, 6, 8 and 10 µg/ml for cilnidipine and 4, 8, 1.2, 1.6 and 20 µg/ml for olmesartan medoximil. The absorbance of resulting solution was measured at 256 nm and 240 nm and calibration curve were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using calibration curve equation. The concentration of CIL and OLM in sample solution was determined by solving the respective simultaneous equation generated by using absorptivity coefficients and absorbance values of CIL and OLM at these wavelengths. The proposed method was validated according to ICH guidelines. [4]

Linearity

The calibration curves were plotted over a concentration range of 2-10 µg/ml for cilnidipine and 2-20 µg/ml for olmesartan medoximil. Accurately measured standard solution of CIL 0.2, 0.4, 0.6, 0.8 AND 1.0 ml and OLM 0.4, 0.8, 1.2, 1.6 and 2.0 ml were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbance of solution was measured at 256 and 240

nm against methanol as blank. The calibration curve was constructed by plotting absorbance versus concentration and the regression equation was calculated.

Method Precision

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solution (n = 6) for CIL and OLM without changing the parameter of proposed Spectrophotometry method.

Intermediate Precision

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 3 different concentration of standard solution of CIL and OLM.

Accuracy

The accuracy of the method was determined by calculating recovery of CIL and OLM by standard addition method. Know amounts of standard solution of CIL and OLM were added at 80, 100, 120 % level of pre quantified sample solution of CIL 4 µg/ml and OLM 8 µg/ml. the amounts of CIL and OLM were estimated by applying obtained values to the respective regression line equation. The experiment was repeated for five times. Result show in table 1

Limit of detection and limit of Quantification

The limit of detection and the limit of quantification of the drug were derived by calculating the signal to noise ratio using the following equation designated by ICH guidelines.

LOD = 3.3 × σ/S

LOQ = 10 × σ/S

Where, σ = the standard deviation of the response.

S = slope of the calibration curve.

Analysis of tablet dosage form

Twenty tablets were weighed, their mean weight determined and finally powdered. An accurately weighed tablet powder equivalent to 10 mg of CIL and 20 mg OLM was transfer into 100 mL volumetric flask containing 50 mL of diluent, sonicate for 10 minute and volume was made up to the mark with diluent, the resulting solution was filtered using 0.45 µm filter. From filtrate, 1 mL of solution was transferred into 100 mL volumetric flask and volume was made up to mark with diluent to obtain the concentration of 10 µg/mL CIL and 20 µg/mL of OLM. Absorbance of the

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resulting solution was measured at 256 nm and 240 nm. Relative concentration of two drugs in the sample solution was calculated using respective simultaneous equation generated by using absorptivity coefficients and absorbance values of CIL and OLM at these wavelengths. Result show in table 2

RESULT AND DISCUSSION

In this method, two wavelengths were used for the analysis of the drugs 256 nm and 240 nm are the wavelengths at which calibration curves were prepared for both drugs. The criteria for obtaining maximum precision by this method calculated. [5] The standard solution of CIL and OLM were scanned in UV range zero order spectra of drugs recorded (**Figure 1**). The R^2 value for CIL was found to be 0.999 at 256 nm, 0.999 at 240 nm and for OLM was found to be 0.999 at 256 nm, 0.999 at 240 nm. The developed method was found to be linear in the concentration range of 2-10 (µg/mL) for CIL and 4-20 (µg/mL) for OLM. % Recovery of CIL and OLM were found to be 99.87% - 100.29% and 99.86% - 100.13% respectively which are within limit (98.0%- 102%) as per ICH guideline. So the developed Method is found to be accurate. The % RSD for CIL was found to be 0.39 at 256 nm and 0.33 at 240 nm and for OLM was found to be 0.29 at 256nm and 0.19 at 240 nm, which is within limit (< 2%). This indicates the developed Method have good repeatability. The % RSD of Intraday precision for CIL was found to be 0.08-0.4 at 256 nm, 0.1-3.0 at 240 nm and for OLM was found to be 0.1 – 0.2 at 256 nm, 0.07- 0.3 at 240 nm The % RSD of Interday precision for CIL was found to be 0.1-0.7 at 256nm, 0.2-0.5 at 240 nm and for OLM was found to be 0.2- 0.4 at 256 nm, 0.27 – 0.60 at 240 nm which is within limit (< 2%).

So the developed Method is found to be precise. The limit of detection (LOD) for CIL was found to be 0.06 µg /mL at256 nm, 0.10 µg /mL at 240 nm and for OLM 0.21 µg /mL at 256 nm, 0.19 µg /mL at 240 nm. The limit of quantification (LOQ) for CIL was found to be 0.20 µg /mL at 256 nm, 0.30 µg /mL at 240 nm and for OLM 0.63µg/mL at 256 nm, 0.58 µg /mL at 240 nm. % Assay of CIL and OLM were found to be 100.03 %w/w and 99.58 %w/w respectively which is within limit. So the developed Method can be applied for the simultaneous estimation of CIL and OLM in tablet dosage form. All the regression analysis data and summary of validation parameters of proposed method is reported in table 3.

CONCLUSION

The proposed spectrophometric method was found to be simple, sensitive, accurate and precise for simultaneous determination of CIL and OLM in tablet dosage form. The method utilize easily available and low cost solvent like methanol for analysis of CIL and OLM hence the method was also found to be economic for estimation of CIL and OLM from tablet. The method can be used for the routine analysis of the CIL and OLM in combined dosage form without any interference of excipients.

ACKNOWLEDGEMENT
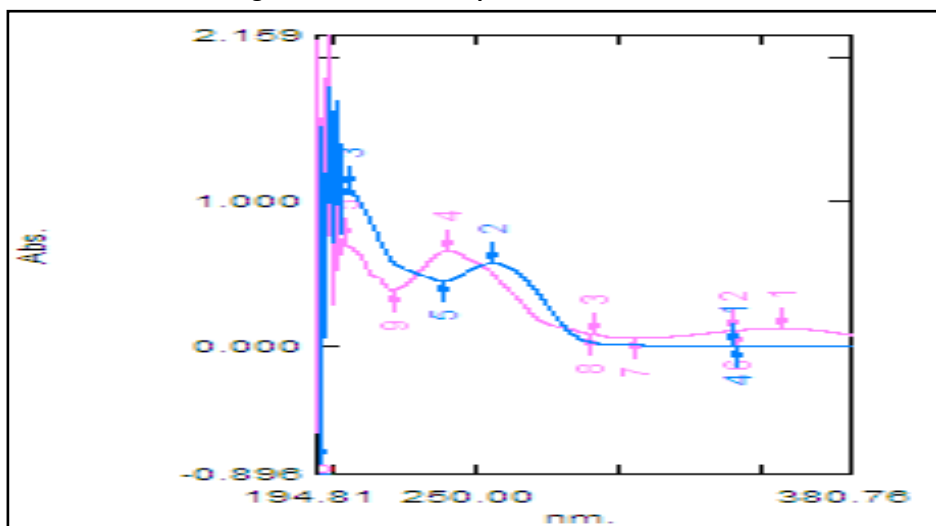
Figure 1 Overlain UV spectra of CIL and OLM

Table 1 Recovery Studies for CIL and OLM by Simultaneous Equation Method.

Conc. of Sample taken (µg /mL)	Level	Conc. of Pure spiked API (µg /mL)	Total Conc. (µg /mL)	Mean Total Conc. Found (n=3) (µg /mL)	%Recovery Mean (n=3)	%RSD
CIL 4	80 %	3.2	7.2	7.22	100.29	0.8
	100 %	4	8	7.99	99.98	1.7
	120 %	4.8	8.8	8.78	99.87	1.6
OLM 8	80 %	6.4	14.4	14.37984	99.86	0.4
	100 %	8	16	16.0208	100.13	0.3
	120 %	9.6	17.6	17.59296	99.96	0.5

Table 2 Assay Result for CIL and OLM by Simultaneous Equation Method

FORMULATION	Tablet formulation	
	CIL	OLM
Concentration (µg /mL)	10	20
Concentration found (µg /mL) (n=3)	10.03 ± 0.239137	19.91 ± 0.801041
%Purity	100.03	99.86

International Journal of Pharmacy and Biological Sciences (e-ISSN: 2230-7605)

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Table 3 Summary of Validation Parameters for CIL and OLM by Simultaneous equation Method

PARAMETERS	CIL		OLM	
Linearity (µg/mL)	2-10		4-20	
%Recovery (%)	99.87% - 100.29%		99.86% - 100.13%	
	256 nm	**240 nm**	**256 nm**	**240 nm**
Precision((%RSD) Repeatability (n=6)	0.39	0.33	0.29	0.19
Intra-day (n=3)	0.08-04	0.1-3.0	0.1 – 0.2	0.07- 0.3
Inter-day (n=3)	0.1-0.7	0.2-0.5	0.2- 0.4	0.27 – 0.60
LOD (µg/mL)	0.067	0.100	0.210	0.192
LOQ (µg/mL)	0.204	0.304	0.638	0.583

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