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Development and Validation of First Order derivative UV Spectrophotometric Method for Simultaneous Estimation of Valsartan and Cilnidipine in Combination

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ABSTRACT

Development of a simple, precise and accurate First order derivative spectrophotometric method for the quantitative determination of Valsartan and Cilnidipine in Combination.

Method: *The method is developed using methanol as a solvent. The stock solution of Valsartan and Cilnidipine was prepared in methanol and subsequent dilutions were done in methanol. The first derivative values were measured at 240nm for VAL and 248nm for CIL. Delta lamda 4 and scale 100 set for convert derivative spectra. At zero crossing point of valsartan (248nm) cilnidipine showed a measurable derivative absorbance where as at zero crossing point of cilnidipine (240) valsartan showed an appreciable derivative absorbance value. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, LOD and LOQ.*

Results: *The drug solutions obeyed Beer–Lambert's law and linearity was studied in the concentration range of 8-48 µg/ml for Valsartan and 1-6 µg/ml for Cilnidipine with correlation coefficient 0.9982 at 240nm and 0.9993 at 248nm. The limit of detection and limit of quantification were found to be 0.083 µg/ml, 0.253 µg/ml for Valsartan and 0.0279 µg/ml, 0.0846 µg/ml respectively. The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 80%, 100% and 120 %. The % recovery was found to be in the range 98.50-101.33%. The low values of % relative standard deviation (RSD) are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The % RSD value less than 2 indicate that the method is precise.*

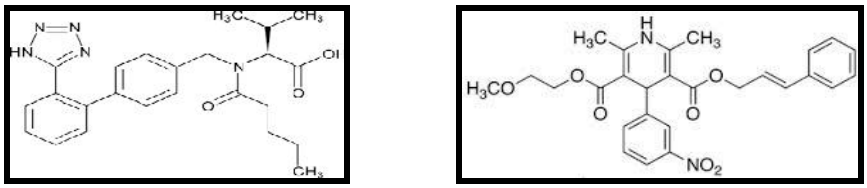
Conclusion: *The above method was a cost-effective quality-control tool for routine analysis of Valsartan and Cilnidipine in Combination.*

Keywords: *Valsartan(VAL), Cilnidipine(CIL), UV Spectrophotometry, First order derivative Spectrophotometry.*

INTRODUCTION

Valsartan[(2S)3methyl2[N({4[2(2H1,2,3,4tetrazol5yl)phenyl]phenyl}methyl)pentanamido]butanoic acid] is Angiotensin Receptor Antagonist selectively inhibits the binding of angiotensin II to AT1 in vascular smooth muscle and the adrenal glands. Valsartan is official in IP and USP [1-2]. Cilnidipine [3-cinnamyl 5-(2-methoxyethyl)2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5dicarboxylate] is Calcium Channel Blocker with inhibitory action on sympathetic neurotransmitter release [3].

Both drugs are formulated together in the form of tablet dosage form for treatment of hypertension. The chemical structure of both drugs [4-6] were shown in figure 1.



(a) (b)

Fig. 1: Chemical structure of Valsartan (a) and Cilnidipine (b)

From literature survey it reveals that various analytical methods have been reported for estimation of Valsartan and Cilnidipine individually or in combination with other drugs either as API or in pharmaceutical dosage form. Literature review also reveals that, there is also no analytical methods reported for estimation of Valsartan and Cilnidipine in bulk and tablet formulation. So the purpose of this work was to develop a simple, accurate and sensitive first order derivative spectrophotometric method for determination of Valsartan and Cilnidipine in combination.

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MATERIALS AND METHODS

Instrument:

The instrument was double beam UV- visible spectrophotometer (Shimadzu, model 1800, software: UV Probe 2.34) having two matched quartz cell with 1 cm path length. Sonication of sample solutions was done using ultrasonic cleaner.

Materials:

Valsartan (VAL) drug sample was procured from Mediwin pharmaceuticals, Ahmedabad and Cilnidipine (CIL) drug sample was gifted by J.B Chemicals and Pharmaceuticals Pvt Ltd, Mumbai, Maharasthra.

Methods:

Preparation of standard stock solution:

The stock solution having 1000µg/ml concentration of VAL and CIL were prepared separately by dissolving accurately

weighed 100mg of both drugs in 100 ml methanol. Further dilutions of standard stock solutions of both drugs were made with methanol to get the working standard stock solutions of 100µg/ml concentration of VAL and CIL.

Method Development (First order derivative):

Selection of scanning range and sampling wavelength:

The standard solutions of VAL and CIL were diluted with methanol individually to get the concentration of 10µg/ml for both and was scanned in UV range 200-400 nm. The λmax of both the drugs were found to be 250nm and 240nm respectively in normal UV spectra shown in figure 2.

Development of first order derivative spectra:

The spectral data was then processed to obtain first order derivative spectrum at wavelength interval of 2nm for the range of 200-400nm. It was observed that VAL shows ZCP at 248nm and CIL shows ZCP at 240nm. At ZCP of VAL (248nm), CIL showed a measurable dA/dλ [7-8] whereas at ZCP of CIL (240nm), VAL showed a measurable dA/dλ. Hence the wavelengths 240nm and 248nm were selected as analytical wavelengths for determination of VAL and CIL first order derivative method respectively shown in figure 3.

Method Validation:

The above proposed method was validated according to ICH Q2 R1 guidelines for validation of analytical procedures [9] in order to determine the linearity, Accuracy, Precision, LOD and LOQ.

Linearity and Range:

Calibration curve constructed was linear over a selected range of 8-48µg/ml for VAL and 1-6µg/ml for CIL. The aliquots of both the drugs used in linearity studies were converted to first derivative spectra and the derivative absorbance at 240nm and 248nm for VAL and CIL were measured respectively. The calibration curve of responses against concentration was plotted was shown in figure 4 and 5. Each concentration was repeated five times. Correlation coefficient and regression line equations for VAL and CIL were calculated and were shown in table no.1.

Accuracy:

The accuracy of the developed method was determined by finding out the amount of recovery of Valsartan and Cilnidipine. For the accuracy standard addition method was used where, as known amount of VAL and CIL were added to the known concentration (16µg/ml VAL and 2µg/ml CIL). The amount recovered was found by measuring the absorbance of the solution and was expressed as mean recovery of samples with upper and lower limits of percent relatives of standard deviation. Recovery was done at three different levels i.e. 80%, 100% and 120%, within the linearity range of both the drugs.

Precision:

Repeatability (n=6):

For the repeatability study, from the working stock solution of both drugs, aliquot of 1.6 ml VAL and 0.2 ml CIL were transferred to a separate 10 ml volumetric flask and diluted upto mark with methanol such that it gives the concentration of 16µg/ml and 2µg/ml of VAL and CIL respectively. The absorbance of the solutions was measured at 240nm and 248nm respectively. The procedure was repeated six times and % RSD was calculated and shown in table no. 3.

Intraday Precision (n=3):

From the working stock solution, aliquots of 2.4ml, 3.2ml and 4ml of VAL and 0.3 ml, 0.4 ml and 0.5 ml of CIL were transferred to separate 10 ml volumetric flask and diluted upto the mark with methanol to give concentration of 24, 32 and 40 µg/ml for VAL, 3, 4 and 5µg/ml for CIL. The solutions were analysed three times on the same day and % RSD was calculated and shown in table no. 3.

Interday Precision (n=3):

From the working stock solution, aliquots of 2.4ml, 3.2ml and 4ml of VAL and 0.3 ml, 0.4 ml and 0.5 ml of CIL were transferred to separate 10 ml volumetric flask and diluted upto the mark with methanol to give concentration of 24, 32 and 40 µg/ml for VAL, 3, 4 and 5µg/ml for CIL. The solutions were analysed three times on three different days and % RSD was calculated and were shown in table no. 3.

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

Limit of detection (LOD) is the minimum concentration of the analyte in the sample which can be analysed by the instrument. Limit of quantification (LOQ) is the minimum concentration of the analyte that can be reliably quantified. The Limit of detection (LOD) and Limit of quantification (LOQ) were measured using following formula. The values of LOD and LOQ for VAL and CIL were shown in table no. 5.

$$LOD = 3.3 \times (SD/Slope)$$
$$LOQ = 10 \times (SD/Slope)$$

Where,

SD = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

Assay of Combination:

Combination containing both Valsartan and Cilnidipine were used for the study. Combine solution equivalent to 80mg of Valsartan and 10mg of Cilnidipine and transferred in to a 100 ml volumetric flask to bring both drugs in 8:1 ratio and stock solution of this was prepared in methanol, sonicated for 15 min, the volume was adjusted up to the mark with same solvent. This stock solution contains Valsartan 800µg/ml and Cilnidipine 100µg/ml. Then the appropriate dilution of 16µg/ml (VAL) and 2µg/ml (CIL) was made using methanol as solvent. All the determinations were carried out in triplicate. The absorbance of the prepared solutions was measured at ZCP of VAL and ZCP of CIL and then the concentration of both the drug was calculated using calibration curve equation. The amount of the drug found in combination was shown in table no. 4.

RESULT AND DISCUSSION

The present paper describes the estimation of VAL and CIL in combination by First order derivative method. The Beer-Lambert's concentration range was found to be 8-48µg/ml and 1-6µg/ml for both drug VAL and CIL at 240 nm and 248 nm respectively. The correlation coefficient was found to be 0.9982 for VAL and 0.9993 for CIL for proposed method. Precision was determined by studying repeatability, intraday and interday precision. The standard deviation and Relative standard deviation (%RSD) were calculated for both the drugs. The % RSD for proposed method were found to be not more than 2.0% which indicates good intermediate precision. The values of LOD and LOQ were 0.083µg/ml and 0.253µg/ml for VAL and 0.027µg/ml and 0.084µg/ml for CIL respectively.

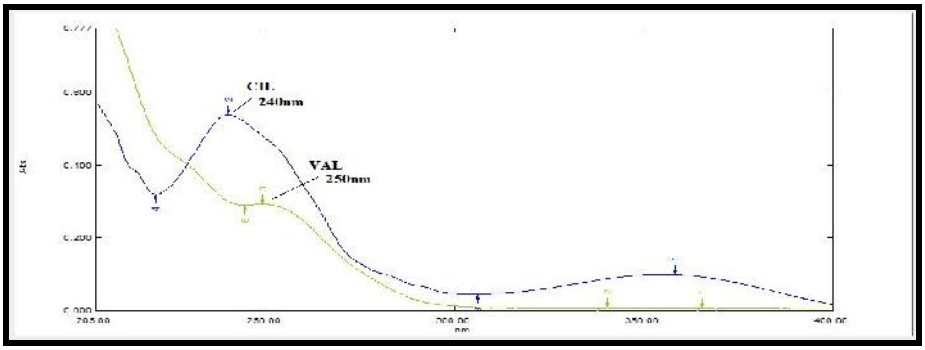

Fig. 2: zero order spectra of VAL(10µg/ml) and CIL(10µg/ml) in methanol.

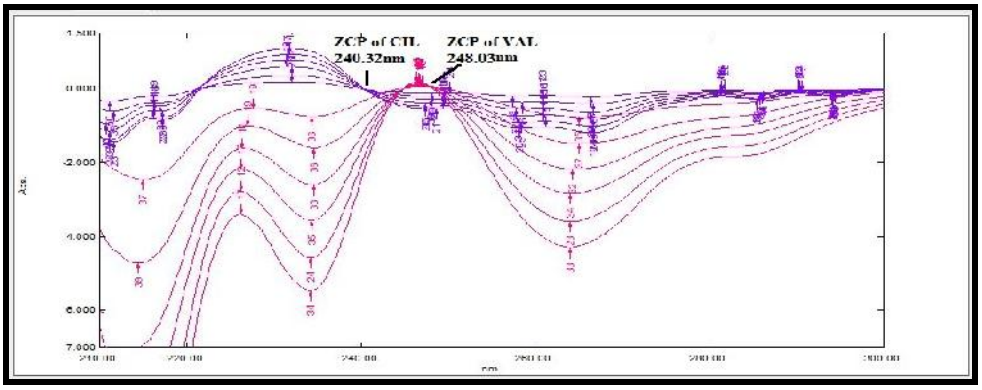

Fig. 3: Overlain first order spectra of VAL and CIL in methanol.

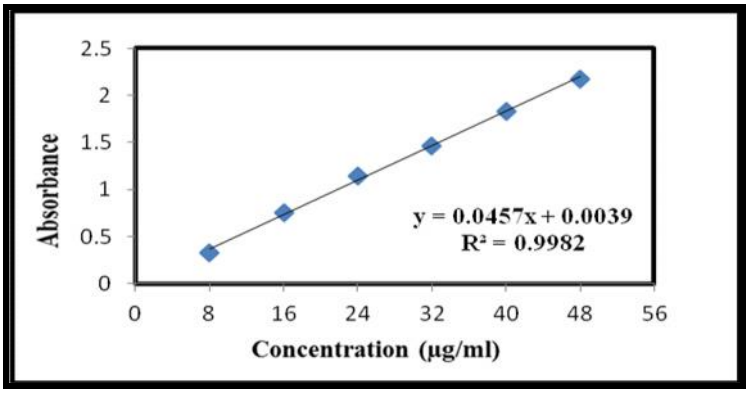

Fig. 4: Linearity graph for first order derivative of VAL.

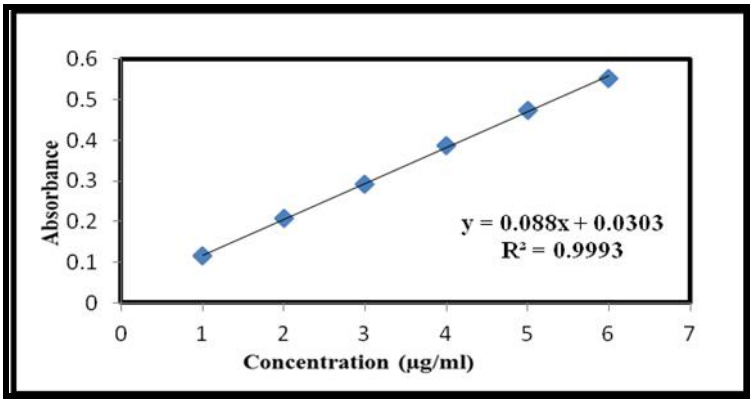

Fig. 5: Linearity graph for first order derivative of CIL.

Table No. 1: Optical Characteristics

Parameters	Valsartan at 240nm	Cilnidipine at 248nm
Beer's law limit (µg/ml)	8 - 48µg/ml	1 - 6µg/ml
Regression equation	y = 0.0457x + 0.0039	y = 0.088x + 0.0303
Slope (m)	0.0457	0.088
Intercept (c)	0.0039	0.0303
Correlation coefficient (R^2)	0.9982	0.9993

Table No. 2: Results of Recovery studies

Drug	Concentration of STD drug	Recovery level(%)	Amount of drug added(µg/ml)	Amount of drug recovered(µg/ml)	%recovery±SD
VAL	16	80	12.8	15.98	99.87±0.226
		100	16	15.99	99.95±0.294
		120	19.2	16.11	100.70±0.405
CIL	2	80	1.6	2.01	99.33±1.040
		100	2	2.02	101.33±0.763
		120	2.4	1.97	98.50±0.500

*SD = standard deviation, *STD = standard

Table No. 3: Repeatability, Inter-day and Intra-day precision of Valsartan and Cilnidipine

Drug	Concentration(µg/ml)	Average ABS±SD	%RSD
REPEATABILITY(n=6)			
VAL	16	0.749±0.00167	0.223
CIL	2	0.208±0.00075	0.361
INTRADAY PRECISION(n=3)			
VAL	24	1.145±0.0005	0.050
	32	1.467±0.0010	0.068
	40	1.833±0.0015	0.083
CIL	3	0.293±0.0010	0.341
	4	0.386±0.0015	0.395
	5	0.474±0.0005	0.121
INTER DAY PRECISION(n=3)			
VAL	24	1.145±0.0010	0.087
	32	1.466±0.0011	0.078
	40	1.831±0.0015	0.083
CIL	3	0.292±0.0005	0.197
	4	0.384±0.0015	0.397
	5	0.474±0.0011	0.243

*SD = standard deviation, ABS = Absorbance

Table No. 4: Analysis of Combination

Drug	Concentration in dosage form (mg/ml)	Concentration taken for assay(µg/ml)	Concentration found	%Assay
VAL	80	16	16.30	101.87%
CIL	10	02	02.01	100.50%

Table No. 5: Limit of detection (LOD) and Limit of Quantification (LOQ)

Parameters	Valsartan	Cilnidipine
LOD (µg/ml)	0.083	0.027
LOQ (µg/ml)	0.253	0.084

CONCLUSION

A simple, accurate and precise UV first order derivative spectrophotometric method has been developed for the estimation of VAL and CIL in combination. It has advantage that it eliminates the spectral interference from one of the two drugs while estimating the other drug by selecting zero crossing point in the derivative spectra of each drug at selected wavelength.

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