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Development and Validation of Second Order Derivative Spectrophotometric Method for Simultaneous Estimation of Cilnidipine and Valsartan in Synthetic Mixture

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ABSTRACT

A simple, accurate, and precise UV spectrophotometric method has been developed and validated for simultaneous estimation of Cilnidipine and Valsartan in synthetic mixture. The estimation of drugs was done by Second Order Derivative spectrophotometric method using 219 nm and 227 nm wavelength. Methanol was used as a solvent. The Linearity was obtained in the concentration range of 2-10 µg/ml for Cilnidipine and 5-25 µg/ml for Valsartan with R^2 0.9994 and 0.9980 respectively. Accuracy was determined by recovery studies and showed % recovery between 98 to 102 % for both the drugs. The LOD and LOQ values of Cilnidipine were found to be 0.33 and 1.01 and for Valsartan values were found to be 0.18 and 0.55. The developed method was validated as per the ICH Guidelines Q2 (R1).

Keywords: Cilnidipine, Valsartan, UV Spectrophotometry, Second Order Derivative spectroscopy.

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INTRODUCTION

Cilnidipine is a dihydropyridine calcium channel blocker. Cilnidipine is chemically, 1,4- Dihydro-2,6-dimethyl- 4-(3-nitrophenyl)-3,5-pyridinecarboxylic acid 2-methoxyethyl(2E)-3-phenyl-propenyl ester. Cilnidipine is a dual blocker of L-type voltage gated calcium channels in vascular smooth muscle and N-type calcium channels in sympathetic nerve terminals that supply blood vessels. Cilnidipine shows first pass metabolism. Valsartan is an angiotensin II receptor antagonist with particular high affinity for type 1 angiotensin receptor. Valsartan is chemically 3-methyl-2-[N-({4-[2-(2H-1,2,3,4-tetrazol- 5yl)phenyl]phenyl}methyl)pentanamido]butanoic acid. Valsartan inhibits the bimding of angiotensin II to AT1 which effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. Synthetic mixture contains 10 mg Cilnidipine and 80 mg Valsartan. Cilnidipine and Angiotensin Receptor Blocker may be more effective than treatment with an Angiotensin Receptor Blocker alone because Cilnidipine converts the Angiotensin 2 that is induced by ARB into Angiotensin 1 and exhibits additive protective effects on renal and cardiac organs.

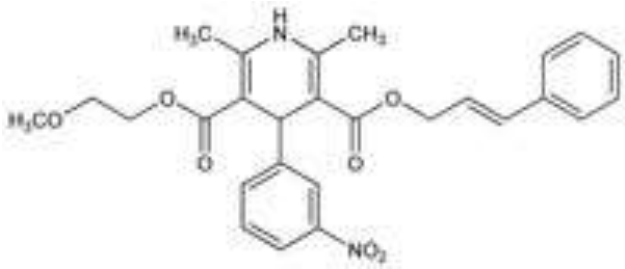

Figure 1: Chemical structure of cilnidipine

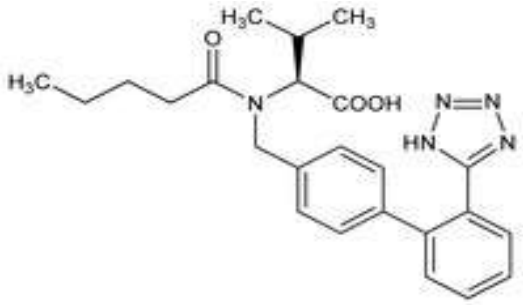

Figure 2: Chemical structure of Valsartan

Based on Literature review, it reveals that there are individual methods available and in combination with other drugs such as UV Spetrocphotometry, HPLC, HPTLC but no method has been developed for the combination of Cilnidipine and Valsartan. So it was worth to develop method for simultaneous estimation of Cilnidipine and Valsartan.

MATERIALS AND METHODS

Instrumentation and Analytical Conditions

SHIMADZU double beam UV-visible spectrophotometer (model 1800) with 1 cm matched quartz cuvettes were used for all absorbance measurements. All weighing were done on electronic analytical balance (Wensar Dab220).Distilled water and Whatmann filter paper (no.41) were used throughout the experimental work.

Software : UV Probe Version 2.31.

Chemicals and Reagents

Reference Standards ofCilnidipine was obtained from the Niksan Pharmaceuticals, Ankleshwar and Valsartan was obtained from the Torrent Pharmaceuticals, Ahmedabad.

Selection of Solvent

Methanol was used as a solvent as both the drugs shows good solubility in methanol.

Preparation of Standard solution of Cilnidipine

Accurately weighed quantity of Cilnidipine 10 mg was transferred in 100 ml volumetric flask and diluted upto mark with the methanol to give the standard solution of 100 µg/ml. Further dilutions were made from the standard solution to obtain the concentration of 2, 4, 6, 8, 10 µg/ml respectively.

Preparation of Standard solution of Valsartan

Accurately weighed quantity of Valsartan 10 mg was transferred in 100 ml volumetric flask and diluted upto mark with the methanol to give the standard solution of 100 µg/ml. Further dilutions were made from the standard solution to obtain the concentration of 5, 10, 15, 20, 25 µg/ml respectively.

Preparation of Standard Mixture solution

Accurately weighed quantity of Cilnidipine 10 mg and Valsartan 80 mg was transferred in 100 ml volumetric flask and diluted upto mark with the methanol to give the standard mixture solution of 100 µg/ml of Cilnidipine and 800 µg/ml of Valsartan.µg/ml.

Preparation of Standard Mixture Sample solution

From the standard mixture solution, pipette out 0.2 ml of the standard mixture solution in 10 ml of volumetric flask and diluted upto the mark with methanol to get concentration of 2 µg/ml and 16 µg/ml for Cilnidipine and Valsartan respectively.

Selection of analytical wavelength

From the standard solution of Cilnidipine and Valsartan, pipette out 0.2 ml and 1.6 ml of solution

of Cilnidipine and Valsartan to get the concentration of 2 µg/ml and 16 µg/ml for Cilnidipine and Valsartan respectively. Both the solutions were scanned in the entire wavelength of 400-200 nm. The zero order overlain spectra of both the drugs was shown in figure. The spectra are converted into second order derivative and both the spectra were overlained. From the second order derivative overlain spectra, both the drugs shows various zero crossing points, but 219 nm was selected as the zero crossing points of Cilnidipine and 227 nm was selected as the zero crossing points of valsartan. At 219 nm (zero crossing points of Cilnidipine), Cilnidipine shows zero absorbance but Valsartan has considerable absorbance while at 227 nm (zero crossing points of Valsartan), Cilnidipine has considerable absorbance. Both this wavelength 219nm and 227 nm was used for the determination of Valsartan and Cilnidipine respectively.

Calibration Curve

A calibration curve was plotted over a concentration range of 2-10µg/ml for Cilnidipine and 5-25 µg/ml for Valsartan. Accurately pipette out standard solution of Cilnidipine (0.2, 0.4, 0.6, 0.8 and 1.0 ml) and standard solution of Valsartan (0.5, 1, 1.5, 2 & 2.5 ml) were transferred to a separate series of 10 ml of volumetric flasks and diluted to the mark with Methanol. The absorbance of each solution of Cilnidipine was measured at the 227 nm and each solution of Valsartan was measured 219 nm respectively. Calibration curves were constructed for Cilnidipineand Valsartan by plotting absorbance versus concentrations at both wavelengths.

Validation of proposed method

Linearity

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in the samples. Cilnidipine was linear with the concentration range of 2-10µg/ml at 227 nm and Valsartan showed the linearity in the range of 5-25 µg/ml at 219 nm. Plot the calibration curve of absorbance Vs concentration and find out correlation coefficient for Cilnidipine and Valsartan and slope and intercept.

Precision

Repeatability

The concentration of 6 µg/ml of Cilnidipine and 15 µg/ml of Valsartan was repeated for six times and the % RSD was determined.

Intraday

Three different concentrations of Cilnidipine (2, 6, 10µg/ml) and of Valsartan (5, 15, 25 µg/ml) was analyzed for three times at different time interval on the same day and the % RSD was determined.

Interday

Three different concentrations of Cilnidipine (2,6,10 µg/ml) and of Valsartan (5, 15, 25 µg/ml) was analyzed for three times at 3 different days and the % RSD was determined.

Accuracy

Accuracry was measured by standard addition technique, performed by addition of known amounts of Cilnidipine and Valsartan to known concentration of the test solution. Recovery studies were carried out at three different levels 80, 100 and 120%. Absorbances of each level was measured for three times.

Limit of detection and Limit of quantification

Limit of detection and Limit of quantification were measured by the following equation as follows:

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

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

RESULTS AND DISCUSSIONS

Derivative spectrophotometric method has been developed for simultaneous estimation of Cilnidipine and Valsartan in synthetic mixture. Cilnidipine shows the good linear relationship over the concentration range of 2-10 µg/ml with a regression coefficient of R^2 0.9994 at 227 nm and Valsartan shows the good linear relationship over the concentration range of 5-25 µg/ml with a regression coefficient of R^2 0.9980 at 219 nm. The precision study was also carried out with the % RSD less than 2 %(< 2 %). The recovery studies were carried out at three different levels. % Recovery of Cilnidipine is obtained between 98.48- 102.28 % whereas for Valsartan it is 98.66-101.72% having % RSD less than 2 %. The LOD and LOQ values for the Cilnidipine is 0.33 and 1.01 and for the Valsartan it is 0.18 and 0.55. the proposed method is validated according to ICH guidelines.

Preparation of Synthetic Mixture

Ingredients	Quantity Taken
Cilnidipine	10 mg
Valsartan	80 mg
HPMC K-15M	140 mg
Ethyl cellulose	35 mg
Magnesium stearate	1.5 mg
Talc	1.5 mg
Lactose monohydrate	82 mg

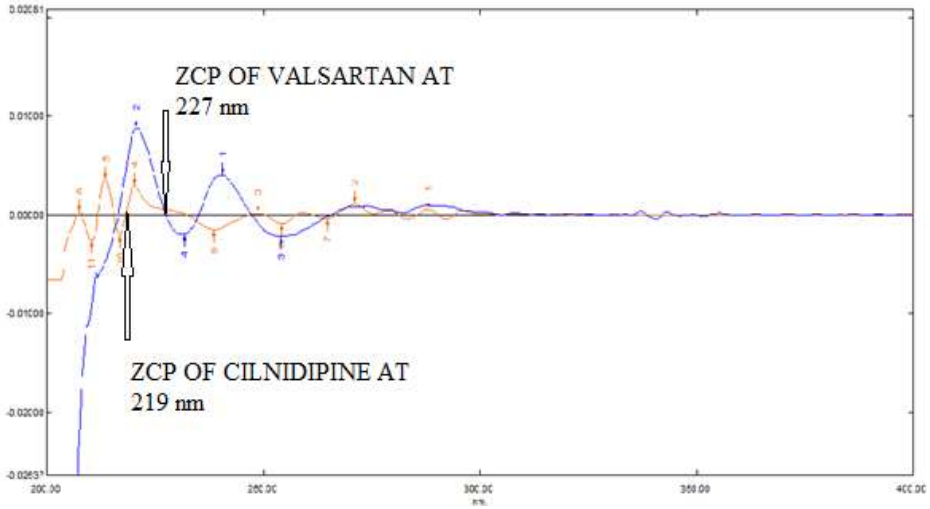

Figure 3: Second order overlain spectra of Cilnidipine and Valsartan

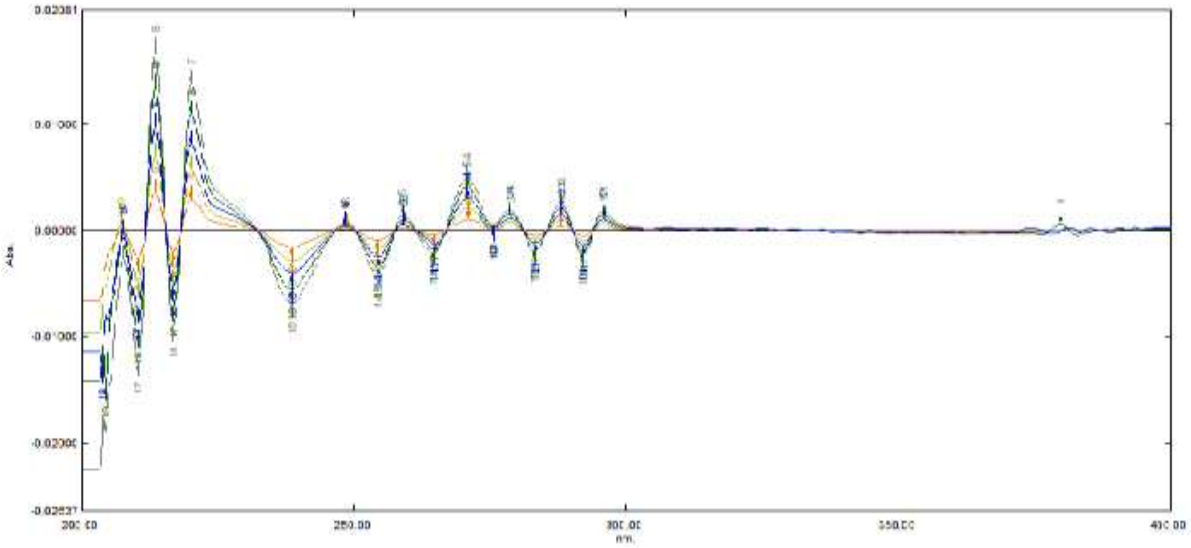

Figure 4: Overlain spectra of Cilnidipine at 227 nm

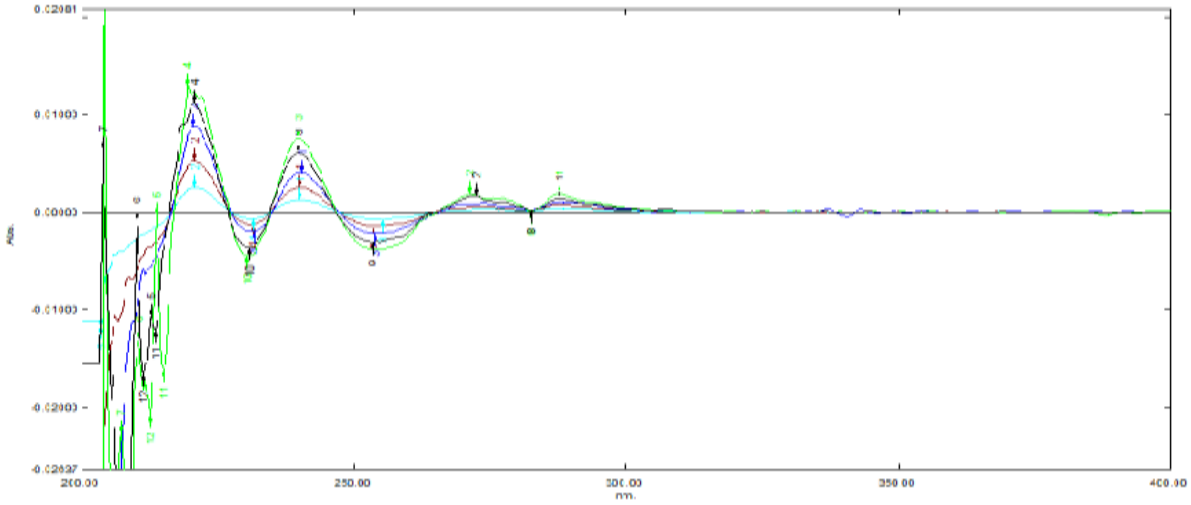

Figure 5: Overlain spectra of Valsartan 219 nm

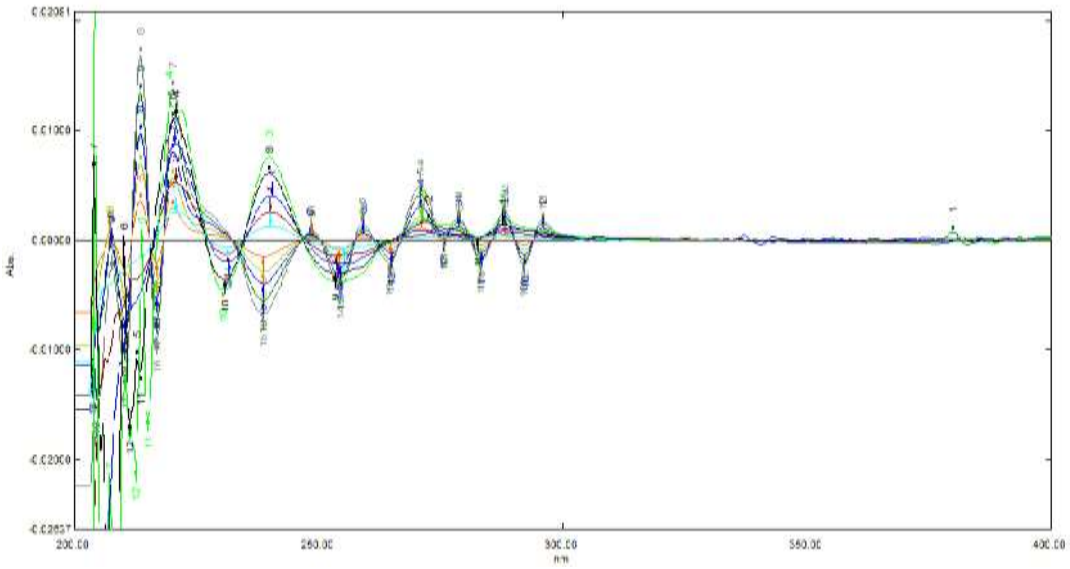

Figure 6: Overlain spectra of Cilnidipine and Valsartan

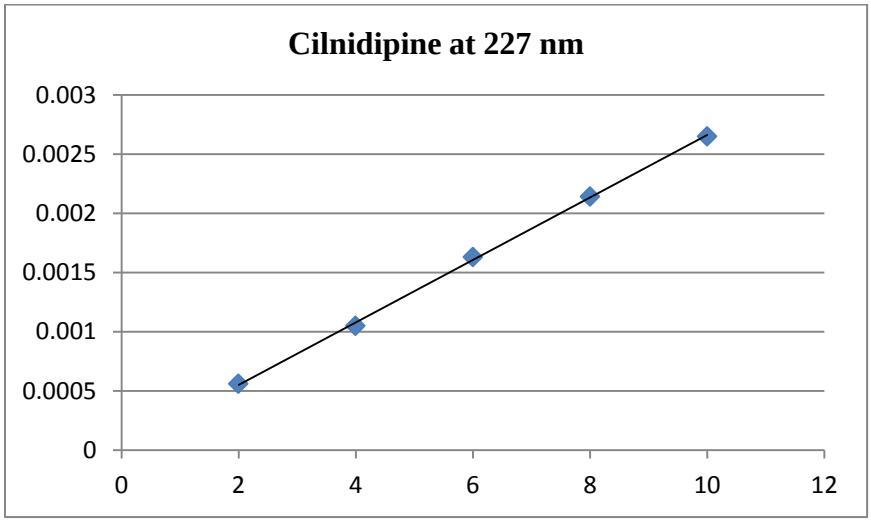

Figure 7: Linearity of Cilnidipine at 227 nm

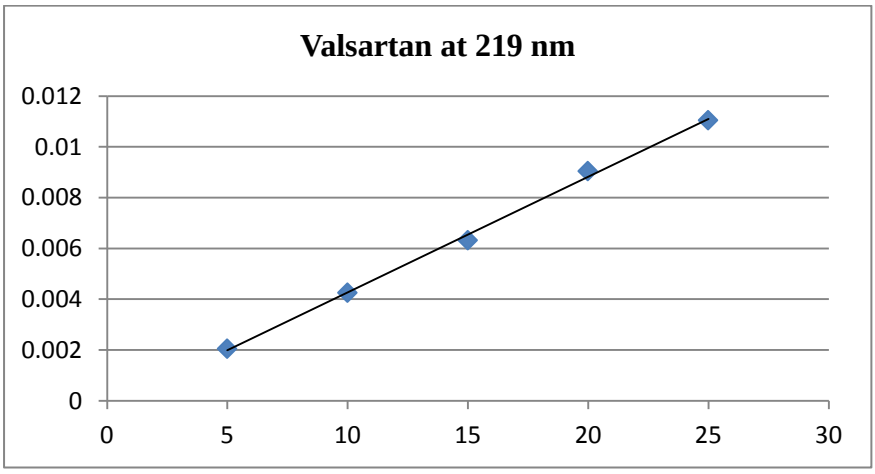

Figure 8: Linearity of valsartan at 219 nm

Table 1: Linearity data for Cilnidipine and Valsartan

Cilnidipine (227 nm)			Valsartan (219 nm)		
Conc (µg/ml)	**Mean absorbance ± SD (n=5)**	**% RSD**	**Conc (µg/ml)**	**Mean absorbance ± SD (n=5)**	**% RSD**
2	0.000556 ±0.000010	1.834	**5**	0.002074 ±0.000024	1.165
4	0.001066 ±0.000016	1.524	**10**	0.004236 ±0.000040	0.951
6	0.001662 ±0.000021	1.284	**15**	0.006334 ±0.000033	0.524
8	0.002168 ±0.000029	1.349	**20**	0.009044 ±0.000014	0.165
10	0.002662 ±0.000019	0.728	**25**	0.011054 ±0.000019	0.177

Recovery studies is within the 10 ppm solution. Total concentration of recovery study is 2.25, 2.5 and 2.75 µg/ml respectively

Table 2: Repeatability data for Cilnidipine and Valsartan

Cilnidipine (227nm)			Valsartan (219 nm)		
Conc (µg/ml)	**Mean absorbance ± SD (n=6)**	**% RSD**	**Conc (µg/ml)**	**Mean absorbance ± SD (n=6)**	**% RSD**
6	0.001638 ±0.000019	1.202	15	0.006338 ±0.000020	0.320

Table 3: Precision for Cilnidipine at 227 nm

Parameters	Concentration(µg/ml)	Mean Absorbance ± S.D (n=3)	% R.S.D
Intraday	**2**	0.000563 ± 0.000004	0.836
	6	0.001643 ±0.000009	0.573
	10	0.002660 ±0.000014	0.530
Interday	**2**	0.000547 ±0.0000081	1.492
	6	0.001607 ±0.000020	1.275
	10	0.002620 ± 0.000020	0.530

Table 4: Precision for Valsartan at 219 nm

Parameters	Concentration (µg/ml)	Mean Absorbance ±S.D (n=3)	% R.S.D
Intraday	**5**	0.002053 ±0.000094	0.454
	15	0.006337 ±0.000012	0.196
	25	0.011050 ±0.000014	0.127
Interday	**5**	0.002027 ±0.000012	0.615
	15	0.006287 ±0.000028	0.456
	25	0.011017 ±0.000020	0.186

Table 5: Recovery studies for Valsartan

Level of concentration	Amount taken (µg/ml)	Amount spiked (µg/ml)	% Recovery ± S.D (n=3)	%R.S.D
80	10	8	100.77 ±0.992371	0.984
100	10	10	99.483 ±1.447212	1.454
120	10	12	100.15 ±1.551279	1.548

Yes, 22 ppm solution follows the linearity because the linearity range for Valsartan is 5-25 µg/ml. So 22 ppm solution follows the beer law.

Table 6: Recovery studies for Cilnidipine

Level of concentration	Amount taken (µg/ml)	Amount spiked (µg/ml)	% Recovery ±S.D (n=3)	%R.S.D
80	1.25	1	99.95 ±1.106737	1.111
100	1.25	1.25	101.83 ±0.555778	0.545
120	1.25	1.5	101.23 ±0.373661	0.369

The total concentration of recovery studies for 80 %, 100% and 120% is 2.25, 2.5 and 2.75 µg/ml respectively. The concentration is within the linearity range 2-10 µg/ml for Cilnidipine.

The above studies has been corrected.

Table 7: Assay study parameter

Cilnidipine			valsartan		
Label claim	**Amount found**	**% assay**	**Label claim**	**Amount found**	**% assay**
2 mg	1.98 mg	99%	16mg	16.2 mg	101.25%

Table 7: Summary of Validation parameter

Derivative Spectrophotometric Method		
Parameters	**Cilnidipine**	**Valsartan**
Wavelength	227 nm	219 nm
Beer's law limit (µg/mL)	2-10	5-25
Regression equation	y = 0.00026x + 0.00002	y = 0.00046x - 0.00030
Correlation coefficient (r^2)	0.9994	0.9980
Standard deviation of the Y- intercepts of the 5 calibration curve	0.0000266	0.00002498
Mean slope of the calibration curves	0.000262	0.00045
Method precision (Repeatability)	1.202	0.320
Intraday Precision	0.530-0.836	0.127-0.454
Interday precision	0.530-1.492	0.186-0.615
LOD	0.33	0.18
LOQ	1.01	0.55

CONCLUSION

The proposed Derivative spectrophotometric method for the simultaneous estimation of Cilnidipine and Valsartan in synthetic mixture have been developed. The developed method has been validated with a good precision and accuracy. This method can be applied for routine analysis of Cilnidipine and Valsartan.

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