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(RESEARCH ARTICLE)

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Development and validation of spectroscopic simultaneous equation method for simultaneous estimation of Azelnidipine and Valsartan in synthetic mixture

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Abstract

A simple, specific, accurate and precise spectrophotometric method was developed and validated for simultaneous estimation of Azelnidipine and Valsartan in Synthetic Mixture. The wavelength of estimation for Azelnidipine was 240.00 nm and for Valsartan was 250.00 nm. Beer's law is obeyed in the concentration range of 2-10µg/ml and 16-80µg/ml and correlation coefficient of 0.9999 and 0.9993 for Azelnidipine and Valsartan respectively. The % recovery for Azelnidipine and Valsartan were found to be 101.2 % - 101.9 % and 101.8 - 101.9 % respectively. Intraday precision of Azelnidipine and Valsartan were found to be 0.06 – 0.24 and 0.08 – 0.27 % RSD and Interday precision were found to be 0.14 – 0.33 and 0.11 – 0.37 % RSD respectively. The proposed method was also evaluated by the Assay of Synthetic mixture containing Azelnidipine and Valsartan. The % Assay was found to be 100.50 ± 0.002 for Azelnidipine and 100.87 ± 0.001 for Valsartan. Validation of proposed methods was carried out according to ICH Q2R1Guidelines. The proposed methods were found accurate and reproducible for routine analysis of both the drugs in synthetic mixture.

Keywords: Azelnidipine; Valsartan; Simultaneous equation method; UV Spectroscopy

1 Introduction

Hypertension is defined as a sustained diastolic blood pressure greater than 90 mm Hg accompanied by an elevated systolic blood pressure (>140 mm Hg). (1) Hypertension results from increased peripheral vascular smooth muscle tone, which leads to increased arteriolar resistance and reduced capacitance of the venous system. Elevated blood pressure is an extremely common disorder. Although many of these individuals have no symptoms, chronic hypertension either systolic or diastolic can lead to congestive heart failure, myocardial infarction, renal damage, and cerebrovascular accidents.

Method for producing pharmaceutical preparation containing calcium antagonist/ angiotensin II receptor antagonist, Patent No. WO201405807A1; French, Japanese, 2014 Hence, there is a scope to develop analytical methods for Azelnidipine and Valsartan in combination.

Literature review reveals that, various analytical methods have been reported for the estimation of Azelnidipine and Valsartan in pharmaceutical formulation and bulk drug include UV spectrophotometric, High-performance liquid chromatography method (HPLC), Stability indicating RP-HPLC method, HPTLC method, TLC method, LC/MS/MS method and UPLC method in individual and/or in combination of other drug.

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Literature review shows that, there is no reported method available for simultaneous estimation of both the drugs in combination. Therefore, it is thought of interest to developed simple, accurate, precise and rapid methods for simultaneous estimation of Azelnidipine and Valsartan in combination.

2 Material and methods

2.1 Instrument and apparatus

Table 1 Lists of Instrument and Apparatus

Component	Model/Software	Manufacturer
Double Beam UV-Visible Spectrophotometer	Shimadzu-2450, UV Probe 2.34	Shimadzu
Analytical Balance	Sartorius (CD2250)	Wensar
Sonicator	-	Trans-o-Sonic
Volumetric Flask	-	Borosil
Pipettes	-	Borosil
Beaker	-	Borosil

2.2 Reagents and material

All the Reagents and Solvents used were of AR or HPLC grades.

Table 2 Working Standard API

Standard	Purpose	Source
Azelnidipine	Analysis	Nikshan Pharmaceutical Pvt. Ltd.
Valsartan	Analysis	Cipla Pharmaceutical Pvt. Ltd.

2.3 Preparation of standard solutions

2.3.1 Standard solution of Azelnidipine (AZL)

- Preparation of stock solution of AZL

An accurately weighed quantity of AZL (10 mg) was transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of AZL (100µg/ml).

2.3.2 Standard solution of Valsartan (VAL)

- Preparation of standard stock solution of VAL

An accurately weighed quantity of VAL (10 mg) was transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of VAL (100µg/ml).

2.3.3 Preparation of Standard Mixture Solution (AZL+ VAL):

0.2 ml of working standard stock solution of AZL (100µg/ml) and 1.6 ml of standard Stock solution of VAL (100µg/ml) were pipetted out into 10ml volumetric flask and volume was adjusted to the mark with methanol to get 2µg/ml of AZL and 16µg/ml of VAL.

2.3.4 Preparation of Test Solution

The preparation of synthetic mixture.

- Azelnidipine: 10 mg
- Valsartan: 80 mg
- Crosscamellose Sodium: 10 mg
- HydroxyPropyl Cellulose: 10 mg
- Hydrated Silicone Dioxide: 10 mg
- Macrogel (PEG) 6000: 30 mg

All the excipients were mixed in 100 ml volumetric flask containing 25 ml of Methanol and Sonicated for 15min. make up the volume with Methanol. The solution was filtered through Whatman filter paper No. 42. (AZL100µg/ml and VAL 800µg/ml)
Finally, the solution had concentration 100µg/ml for AZL and 800µg/ml for VAL from that pipette out 2ml in 100ml volumetric flask and make up to the mark with Methanol.

2.4 Simultaneous equation method

To determine wavelength for measurement, standard spectra Azelnidipine and Valsartan was scanned between 200-400 nm against Methanol. The method was based on the measurement of absorbance of Azelnidipine and Valsartan at 240.00nm and 250.00nm respectively. This method obeyed Beer's law in the concentration range of 2-10µg/ml for Azelnidipine and 16-80µg/ml for Valsartan.

$$Cx = A_2ay_1 - A_1ay_2 / ay_1ax_2 - ay_2ax_1$$

$$Cy = A_1ax_2 - A_2ax_1 / ay_1ax_2 - ay_2ax_1$$

Where,

C_x = Concentration of Valsartan
C_y = Concentration of Azelnidipine
A_1 = Absorbance of test at λ_1 (λ_{max} of AZL)
A_2 = Absorbance of test at λ_2 (λ_{max} of VAL)
ax_1 = Absorptivity of x drug (AZL) at λ_1
ax_2= Absorptivity of x drug (AZL) at λ_2
ay_1 = Absorptivity of y drug (VAL) at λ_1
ay_2= Absorptivity of y drug (VAL) at λ_2

2.5 Spectrophotometric condition

Table 3 Spectrophotometric conditions for Spectroscopic Method

Mode	**Spectrum**
Scan Speed	Fast
Wavelength Range	400-200 nm
Slit width	1 nm
Scaling Factor	10

2.6 Preparation of calibration curve

2.6.1 Calibration Curve for Azelnidipine

This series consisted of five concentrations of standard AZL solution ranging from 2 to 10µg/ml. The solutions were prepared by pipetting out Standard AZL stock solution (100µg/ml). Then pipetting out (0.2ml, 0.4ml, 0.6ml, 0.8ml, and 1.0ml) was transferred into a series of 10 ml volumetric flask and volume was adjusted up to mark with methanol. A zero-order spectrum of the resulting solution was recorded, measured the absorbance at 240.00 nm against a reagent blank solution (methanol).

2.6.2 Calibration Curve for Valsartan

This series consisted of five concentrations of standard VAL solution ranging from 16 to 80µg/ml. The solutions were prepared by pipetting out Standard VAL stock solution (1.6ml, 3.2ml, 4.8ml, 6.4ml, and 8.0ml) was transferred into a series of 10 ml volumetric flask and volume was adjusted up to mark with methanol. A zero-order spectrum of the resulting solution was recorded, measured the absorbance at 250.00 nm against a reagent blank solution (methanol).

2.7 Validation of proposed method

2.7.1 Linearity and range

The linearity response was determined by analyzing 5 independent levels of concentrations in the range of 2 to 10µg/ml for Azelnidipine and 16 to 80µg/ml for Valsartan. (n=6).

2.7.2 Precision

Intraday Precision

The precision of the developed method was assessed by analysing samples of the same batch in nine determinations with three Standard solutions containing concentrations 2, 4, 6µg/ml for AZL and 16,32,48 for VAL and three replicate (n=3) each on same day. Zero order absorbance (D1) was measured at 240.00nm for AZL and 250.00nm for VAL. The %RSD value of the results corresponding to the absorbance was expressed for intra-day precision.

Interday Precision

The precision of the developed method was assessed by analysing samples of the same batch in nine determinations with three Standard solutions containing concentrations 2, 4, 6µg/ml for AZL and 16,32,48 for VAL in triplicate (n=3) per day for consecutive 3 days for inter-day precision. Zero order absorbance (D1) was measured at 240.00 nm for AZL and 250.00nm for VAL. The %RSD value of the results corresponding to the absorbance was expressed for inter-day precision.

2.8 Accuracy

It was determined by calculating the recovery of AZL and VAL by standard addition method.

Solution-A (assay preparation):10µg/ml AZL + 80µg/ml VAL (100µg/ml)
Solution-B (AZL): 100µg/ml
Solution-C (VAL): 100µg/ml

Assay preparation + 80% spike	0.2 ml A + 0.16ml B + 1.28 ml C
Assay preparation +100% spike	0.2 ml A + 0.2 ml B + 1.6 ml C
Assay preparation +120% spike	0.2 ml A + 0.24 ml B + 1.92 ml C

2.9 LOD and LOQ

The Limit of detection and quantitation of the developed method was assessed by analysing 10 replicates of standard solutions containing concentrations 2µg/ml for AZL and 16µg/ml for VAL.

2.9.1 LOD

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD was calculated out by using following formula:

$$DL = 3.3\ \sigma / S$$

Where,

σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

2.9.2 LOQ

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOQ was calculated out by using following formula:

$$DL = 10\ \sigma\ /S$$

2.10 Robustness & ruggedness

Robustness and Ruggedness of the method was determined by subjecting the method to slight change in the method condition, individually, the:

- Change in Wavelength (240±0.2nm and 250±0.2nm)
- Change in instrument (UV-Vis Spectrophotometer model 1800 and 2450),

Three replicates were made for the concentration (2, 4, 6µg/ml of AZL and 16, 32, 48µg/ml of VAL) with different stock solution preparation and the recording of absorbance were done on both the UV-Vis spectrophotometer.

% RSD was calculated.

2.11 Assay by UV spectrophotometric method

Composition of synthetic mixture

- Azelnidipine:10 mg
- Valsartan: 80 mg
- Crosscamellose Sodium: 10 mg
- HydroxyPropyl Cellulose: 10 mg
- Hydrated Silicone Dioxide: 10 mg
- Macrogel (PEG) 6000: 30 mg

All the excipients were mixed in 100 ml volumetric flask and Sonicate for 15min. make up the volume with Methanol. The solution was filtered through Whatman filter paper No. 42.

Finally, the solution had concentration 100µg/ml for AZL and 800µg/ml for MET. From that pipette out 0.2 ml in 100 ml volumetric flask and volume was made up to mark with Methanol to obtain final solution containing 2 µg/ml of AZL and 16 µg/ml of VAL. A zero-order spectrum of the resulting solution was recorded and measure the absorbance at 240.00 nm and 250.00 nm were noted for estimation of AZL and VAL, respectively. The concentrations of AZL and VAL in formulation were determined using the corresponding calibration graph.

3 Results and discussion

The methods were validated with respect ICH Q_2R_1 guidelines.

3.1 Validation of Proposed Spectrophotometric Method for Simultaneous Method

3.1.1 Linearity and range

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 2-10µg/ml for Azelnidipine and 16-80µg/ml for Valsartan. (n=6).

Linearity of azelnidipine

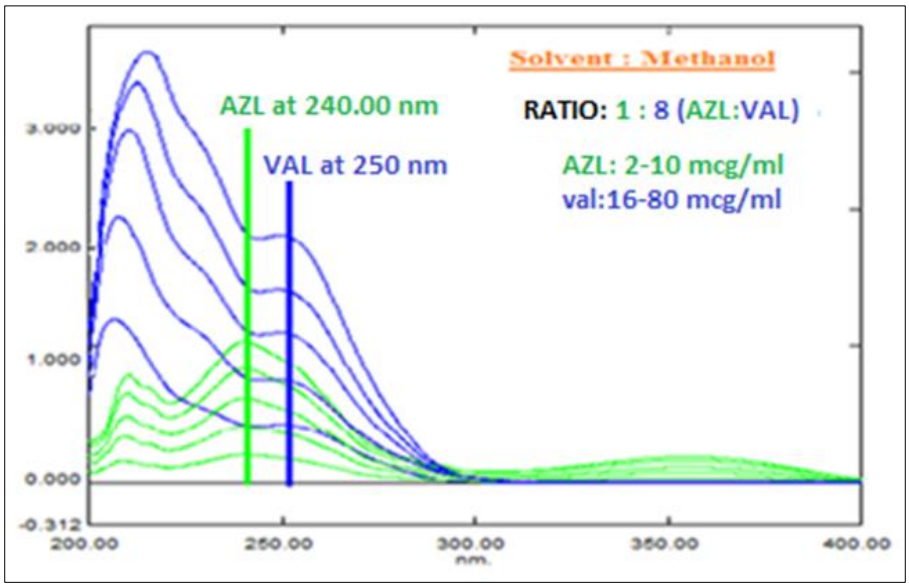

Figure 1 Overlain zero order spectra of AZL and VAL (1:8) ratios, respectively

Table 4 Calibration data for AZL and VAL at240.00nm and 250.00nm respectively. *(n=6)

AZL			VAL		
Conc. µg/ml	**Mean Abs.* ±S.D. At 240nm**	**Mean Abs.* ±S.D. At 250nm**	**Conc. µg/ml**	**Mean Abs.* ±S.D. At 240nm**	**Mean Abs.* ±S.D. At 250nm**
2	0.202±0.001	0.173±0.001	16	0.419±0.002	0.421±0.001
4	0.408±0.003	0.363±0.002	32	0.866±0.001	0.842±0.001
6	0.611±0.003	0.534±0.002	48	1.332±0.001	1.304±0.002
8	0.824±0.002	0.723±0.001	64	1.842±0.001	1.792±0.001
10	1.031±0.016	0.902±0.001	80	2.324±0.002	2.262±0.001

Table 5 Average of absorptivity at 240nm and 250nm

at 240nm		**at 250nm**	
ax1	**0.10238**	**ax2**	**0.08943**
ay1	0.02778	ay2	0.02723

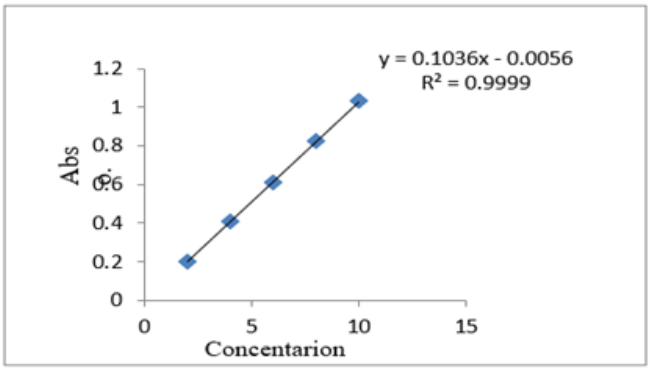

Figure 2 Calibration curve for AZL at 240.00nm

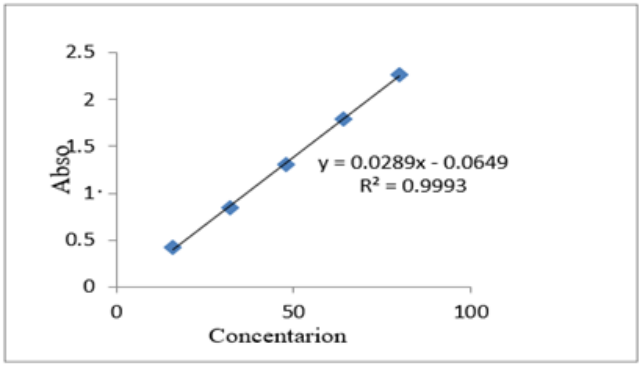

Figure 3 Calibration curve for VAL at 250.00 nm

Table 6 Average of absorptivity at 240nm and 250nm

SR.NO	CONCENTRATION IN MIXTURE (AZL:VAL) (1:8)µg/ml	ABSORBANCE (240nm)	ABSORBANCE (250nm)
1	1:8	0.304	0.279
2	2:16	0.605	0.550
3	3:24	0.840	0.785
4	4:32	1.061	1.026
5	5:40	1.330	1.261
6	6:48	1.739	1.554
7	7:56	2.013	1.867

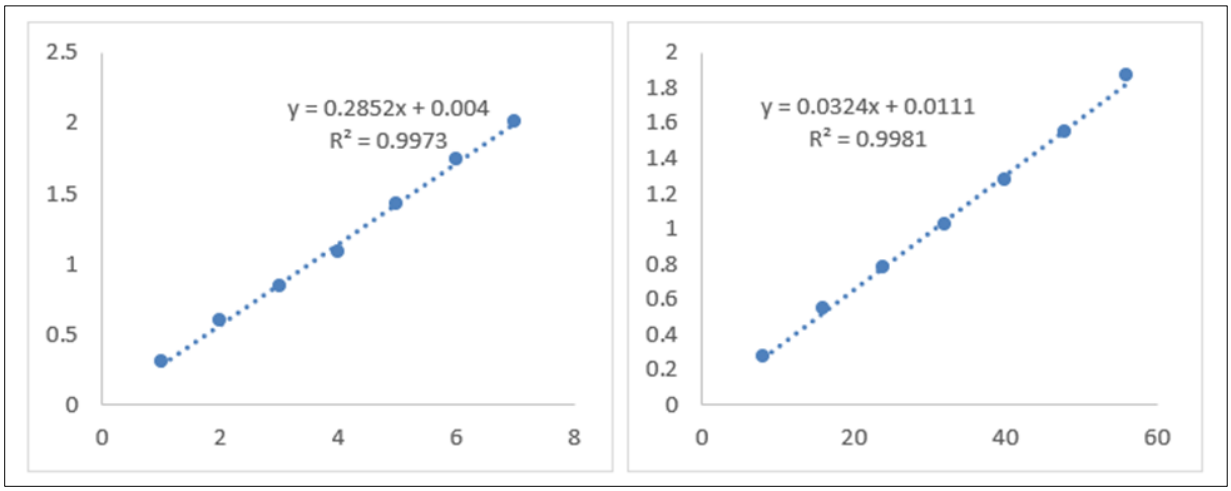

Figure 4 Calibration curve for AZL and VAL in combination

3.2 Precision

3.2.1 Intraday precision

% RSD was found to 0.06 to 0.24 % for Azelnidipine and 0.08 to 0.27 % for Valsartan.

Table 7 Intraday precision data for estimation of AZL and VAL *(n=3)

Conc. (µg/ml)		AZL Abs.* ± S.D.	%RSD	VAL Abs.* ±S.D.	%RSD
AZL	VAL				
2	16	0.613±0.001	0.24	0.552±0.001	0.27
4	32	1.059±0.002	0.19	1.012±0.001	0.15
6	48	1.791±0.001	0.06	1.781±0.001	0.08

3.2.2 Interday precision

% RSD was found to 0.14 to 0.33 % for Azelnidipine and 0.11 to 0.37 % for Valsartan.

Table 8 Interday precision data for estimation of AZL and VAL *(n=3)

Conc. (µg/ml)		AZL Abs.* ± S.D. ±% RSD TZN	%RSD	VAL Abs.* ± S.D.	%RSD
AZL	VAL				
2	16	0.612±0.002	0.33	0.557±0.002	0.37
4	32	1.061±0.002	0.23	1.013±0.002	0.19
6	48	1.794±0.002	0.14	1.784±0.002	0.11

3.3 Accuracy

Accuracy of the method was confirmed by recovery study from Synthetic mixture at three levels. The % recovery was found within 101.2% to 101.9% and 101.8 to 101.9% for Azelnidipine and Valsartan respectively.

Table 9 Recovery data of AZL *(n=3)

Conc. of AZL from formulation (µg/ml)	Amount of Std. AZL added (µg/ml)	Total amount of AZL (µg/ml)	Total amount of AZL found (µg/ml) Mean* ± SD	% Recovery* (n=3)	% RSD AZL
2	-	2.0	2.01±0.001	100.5%	0.15
2	1.6	3.6	3.65 ± 0.001	101.9%	0.12
2	2.0	4.0	4.01 ± 0.001	101.2%	0.20
2	2.4	4.4	4.41 ± 0.002	101.8%	0.10

Table 10 Recovery data of VAL *(n=3)

Conc. of VAL from formulation (µg/ml)	Amount of Std. VAL added (µg/ml)	Total amount of VAL (µg/ml)	Total amount of VAL found (µg/ml) Mean* ± SD	% Recovery* (n=3)	% RSD VAL
16	-	16.00	16.15±0.001	100.93%	0.10
16	12.8	28.8	29.27±0.002	101.9%	0.03
16	16	32.0	32.46±0.010	101.8%	0.12
16	19.2	35.2	35.77±0.001	101.9%	0.11

3.4 LOD and LOQ

Table 11 LOD and LOQ data of AZL and VAL *(n=10)

Conc. (µg/ml)		Avg. abs* ± SD (240.00nm) AZL	% RSD	Avg. abs*±SD (250.00nm) VAL	% RSD
AZL	VAL				
2	16	0.616±0.0017	0.27	0.557±0.0011	0.20
LOD (µg/ml)		0.054		0.136	
LOQ (µg/ml)		0.165		0.414	

3.5 Robustness & ruggedness

Table 12 Robustness and Ruggedness data of AZL and VAL *(n=3)

Condition	Conc. (µg/ml)	Different Instrument				Change in Wavelength 240.00±0.2nm and 250.00±0.2nm			
		UV- 2450	%RSD	UV- 1800	%RSD	240.20 nm	%RSD	239.80 nm	%RSD
AZL Mean (n=3) ± % RSD	2	0.608±0.001	0.16	0.609±0.001	0.25	0.604±0.001	0.16	0.605±0.001	0.25
	4	1.059±0.001	0.09	1.062±0.002	0.18	1.053±0.001	0.14	1.058±0.001	0.10
	6	1.797±0.002	0.11	1.802±0.002	0.11	1.793±0.002	0.14	1.793±0.001	0.08
						250.20nm		249.80nm	
VAL Mean (n=3) ± %RSD	16	0.554 ±0.002	0.36	0.557 ±0.002	0.45	0.553± 0.002	0.36	0.555±0.001	0.27
	32	1.027 ±0.001	0.14	1.028±0.001	0.14	1.023 ± 0.001	0.14	1.029±0.001	0.09
	48	1.232±0.002	0.20	1.234±0.003	0.28	1.231± 0.001	0.81	1.233±0.001	0.12

3.5.1 Assay by UV spectrophotometric method for simultenous method

Table 13 Analysis data of Synthetic Mixture *(n=3)

Formulation (synthetic mixture)		% Assay AZL±SD	%RSD	% Assay VAL±SD	%RSD
AZL	VAL				
2	16	100.50±0.002	0.38	100.87±0.001	0.24

Table 14 Summary of Validation Parameter

Sr. No.	Parameter	Azelnidipine	Valsartan
1	Wave length Max.	240.00nm	250.00nm
2	Linearity (µg/ml) (n=6)	2 to 10 µg/ml	16 to 80 µg/ml
3	Regression equation	y = 0.103x - 0.005	y = 0.028x - 0.064
4	Correlation coefficient (r2)	0.9999	0.9993
5	Accuracy(%Recovery) (n=3)	101.2-101.9%	101.8-101.9%
6	Precision		
	Intra-day (%RSD) (n=3)	0.06-0.24	0.08-0.27
	Inter-day (%RSD) (n=3)	0.14-0.33	0.11-0.37
7	LOD (µg/ml) (n=10)	0.054	0.136
8	LOQ (µg/ml) (n=10)	0.165	0.414
9	Robustness and Ruggedness (%RSD)	0.08-0.25	0.09-0.81
10	Assay	100.50%	100.87%

4 Conclusion

A simple, specific, accurate and precise spectrophotometric method was developed and validated for simultaneous estimation of Azelnidipine and Valsartan in Synthetic Mixture. The wavelength of estimation for Azelnidipine was 240.00 nm and for Valsartan was 250.00 nm. Beer's law is obeyed in the concentration range of 2-10µg/ml and 16-80µg/ml and correlation coefficient of 0.9999 and 0.9993 for Azelnidipine and Valsartan respectively. The % recovery for Azelnidipine and Valsartan were found to be 101.2 % - 101.9 % and 101.8 - 101.9 % respectively. Intraday precision of Azelnidipine and Valsartan were found to be 0.06 – 0.24 and 0.08 – 0.27 % RSD and Interday precision were found to be 0.14 – 0.33 and 0.11 – 0.37 % RSD respectively. The proposed method was also evaluated by the Assay of Synthetic mixture containing Azelnidipine and Valsartan. The % Assay was found to be 100.50 ± 0.002 for Azelnidipine and 100.87 ± 0.001 for Valsartan. Validation of proposed methods was carried out according to ICH Q2R1Guidelines.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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