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ANALYTICAL METHOD DEVELOPMENT AND VALIDATIONs FOR SIMULTANEOUS ESTIMATION OF ANTIHYPERTENSIVE DRUGS

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ABSTRACT

The present work involves development and validation of RP-UPLC methods for simultaneous estimation of Telmisartan and Azelnidipine. In UPLC method, C 18 column (250mm x 4.6mm, 5µm) column was used as stationary phase and Mobile phase A consist of (0.1% formic acid in water as an aqueous phase) and Mobile Phase B consist of (acetonitrile as an organic modifier). The flow rate was 0.8ml/min and both drugs were quantified at 260 nm. The retention time for Telmisartan and Azelnidipine was found to be 5.950 min and 7.293min respectively. The linearity range obtained for RP-UPLC method was 10-160 µg/ml and 5-80 µg/ml for Telmisartan and Azelnidipine respectively. The method was validated according to the guidelines of International Conference on Harmonization (ICH).

KEYWORDS: Telmisartan, Azelnidipine, Validation, UPLC.

INTRDUCTION

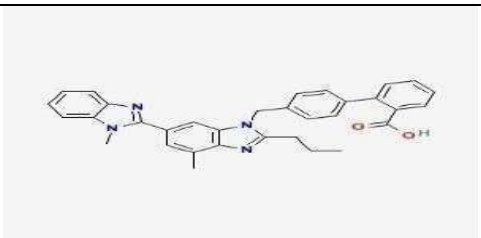
High blood pressure, also known as hypertension, is a common condition that is generally characterized by a higher amount of pressure in blood vessels than the normal value.[1]

Hypertension is one of the main gamble factors for both coronary supply route illness and cardiovascular infection.[2] High blood pressure (BP), otherwise called hypertension, as a clinical bloodpressure of 140/90mmHg or higher affirmed by a subsequent ambulatory blood pressure (or then again home blood pressure checking normal) of 135/85 mmHg or higher. Hypertension is a critical risk factorfor cardiovascular infection.[3]

UPLC is a modern technique which gives a new direction for liquid chromatography. UPLC refers to ultra-performance liquid chromatography, which enhance mainly in three areas: "speed, resolution and sensitivity. In twenty first centenary pharmaceutical industries are focusing for new ways to in economy and shorten time for development of drugs In UPLC main advantage is better efficiency with speedy analysis and this achieved by only smaller particle size. UPLC analysis improves in three areas of Produced Chromatogram with resolved peak. Fast analysis.[4]

Table no. 1.

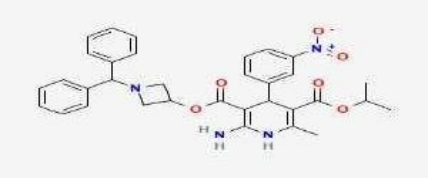
a) Telmisartan.

Structure	
Chemical name	4'-[(1,7'-dimethyl-2'-propyl-1H,3'H-2,5'- bibenzimidazol-3' yl)methyl]bipheny l- 2-carboxylic acid
Category	Anti-hypertensive drug
Molecular Weight	514.617 g/mol
Molecular Formula	C33H30N4O2
Melting Point	261 to 263°C
Solubility	Very soluble in n dimethyl acetamide; sparingly solublein methanol; freely solublein a acetonitrile, practically

	insoluble in water.
pKa (Strong Acid)	3.65
pKa (Strong Basic)	4.1

Table no. 2.

b) Azelnidipine.

Structure	
Chemical name	etidin-3-yl) 5-isopropyl 2- amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
Category	Anti-hypertensive drug
Molecular weight	582.6g/mol
Molecular formula	C_{33} $H_{34}N_4O_6$
Melting point	108℃-113°C
Solubility	Oluble in N Dimethyl acetamide; sparinglysoluble in methanol; freely soluble in acetone, practically insoluble in water
pKa (Strong Acid)	7.16
pKa (Strong Base)	5.18

MATERIALS AND METHODS

Reagents and Chemicals

Telmisartan standard drug was obtained as a gift sample from yarrow Chemical Pvt Ltd., Mumbai and Azelnidipine was Purchased from Yarrow chemicals Pvt. Ltd., Mumbai. Acetonitrile (HPLC grade), Methanol (HPLC grade) from S D Finechem Pvt.Ltd and Formic Acid (AR grade) from Rankemchemicals, Mumbai and Millipore water from inhouse.

Instrumentation and chromatographic condition

The chromatographic separation was performed on Waters AcquityHclass UPLC model, integrated with PDA detector. The analytical Eclipse plus C18 (250mm × 4.6 mm, particle size is 5 µm) for separation. Mobile phase A consist of (0.1% formic acid in water as an aqueous phase) and Mobile Phase B consist of (acetonitrile as an organic modifier) in the ratio 80: 20, (v/v). The flow rate was 0.8ml/min and both drugs were quantified at 260 nm. The injection volume was 20 µl and the column temperature was maintained as 40 °C. Chromotogram is shown in (Fig 1 and 2)

Preparation of mobile phase and standard solution

The Mobile Phase is made up of 0.1% Formic acid in Water as an aqueous phase (A) and Acetonitrile as an Organic modifier (B). It was then filtered through 0.45 µm membrane filter. Finally, the mobile phase was sonicated for 20 min to degas it.

Preparation of diluents

0.1% of Formic Acid and acetonitrile were mixed in the ratio of 80: 20 (% v/v).

Standard preparation Stock solution of Telmisartan

By dissolving 0.1g of pure telmisartan drug in 10ml of methanol solution (100mg/ml),the stock solution (A) was prepared and sonicated for 10 mins. The standard solution (B) were prepared by adding 1ml sol(A) and diluting with 10ml of methanol (1000µg/ml) and series of dilution were made to obtain concentration of 10 - 160 µg/ml with the required dilution of the stock standard solution of Telmisartan with diluents.

Stock solution of Azelnidipine

By dissolving 0.1g of pure Azelnidipine drug in 10ml of methanol solution (100mg/ml),the stock solution (A) was prepared and sonicated for 10 mins. The standard solution (B) were prepared by adding 1ml sol(A) and diluting with 10ml of methanol (1000µg/ml) and series of dilution were made to obtain concentration of 5-80 µg/ml with the required dilution of the stock standard solution of Azelnidipine with diluents.

Preparation of working standard for APIs (Telmisartan and Azelnidipine)

The series of dilution were made to get concentration range 10,20,40,80&160µg/ml of Telmisartan by taking 10,20,40,80&160ml from the stock-B solution of (1000µg/ml) and 5,10,20,40&80 µg/ml of Azelnidipine by taking 5,10,20,40&80 ml from the stock-B of (1000µg/ml) to 10ml volumetric flask respectively, the volume is made up with Diluent and name them as A, B, C, D, E, F. The solutions were sonicated for 20minutes.

Method validation parameters

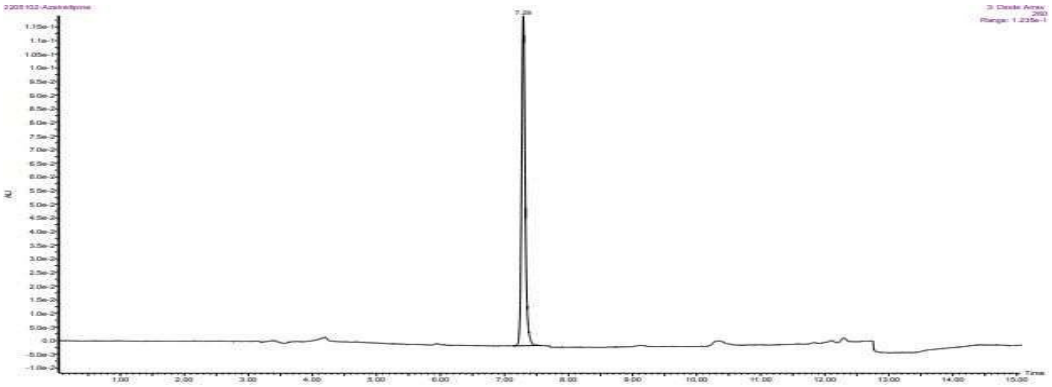
Accuracy Precision Linearity Stability
Limit of detection Limit of quantitation Range
Robustness.

RESULT AND DISCUSSION

Method development

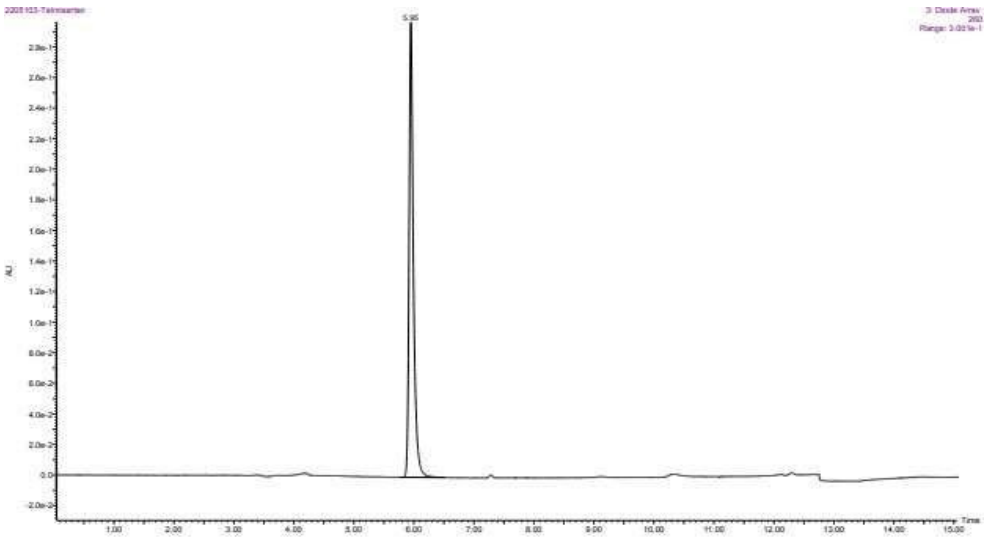
The simultaneous estimation of Telmisartan and Azelnidipine was done by RP-UPLC and mobile phase

consists of (800 volumes of (0.1% formic acid in water as an aqueous phase) and 200 volumes of Water with Acetonitrile. Then finally filtered using 0.45µ membrane filter paper and degassed in sonicator for 20minutes. The detection is carried out using PDA detector at 260nm. The solutions are following at the constant flow rate of 0.8ml/min. The retention time for Telmisartan and Azelnidipine was 5.950 and 7.293minutes respectively. Linearity ranges for Telmisartan and Azelnipine were 10- 160 µg/mL and 5-80 µg/mL respectively and the results were found for in the acceptable as (R^2) = 0.999 and 0.999 for Telmisartan and Azelnidipine respectively. LOD were 0.3 and 0.4 µg/ml and LOQwere 1.0 and 1.3 µg/mL for Telmisartan and Azelnidipine respectively. The all-parameters value of RSD is less than 2.0% indicating the accuracy and precision of the method. The percentage recoverieswere found 99.4- 100.3% and 99.8-100.4% for Telmisartan and Azelnidipine respectively.



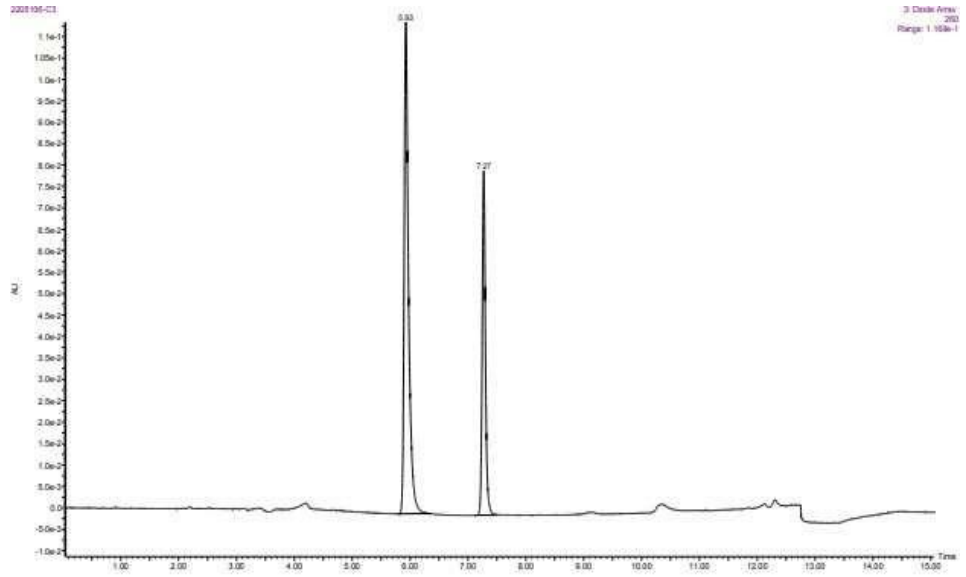
Time	Height	Area	Area%
7.293	120897	7855.314	100.00

Chromatogram of Azelnidipine



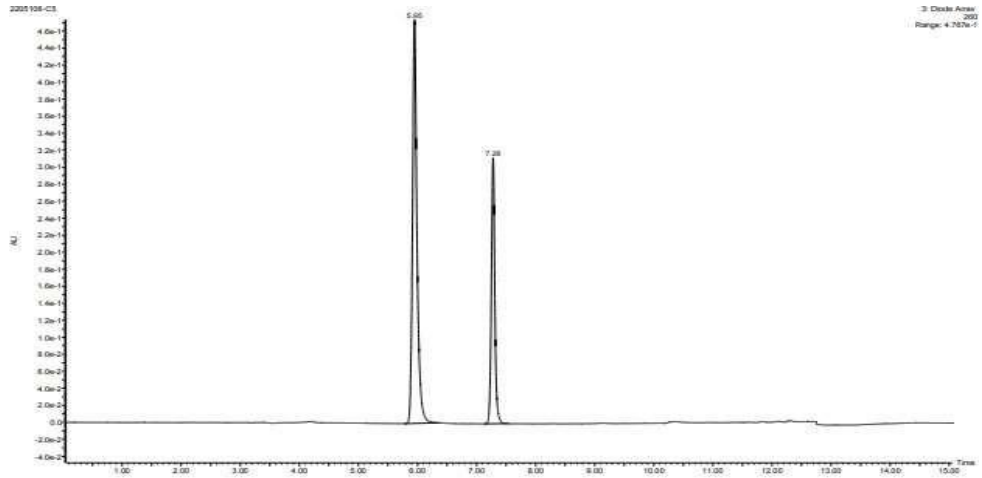
Time	Height	Area	Area%
5.950	297546	24915.510	100.00

Chromatogram of Telmisartan



Time	Height	Area	Area%
5.927	114658	10007.745	65.932
7.273	80307	5171.147	34.068

Chromatogram of Azelnidipine &Telmisartan (5/10) µg/ml

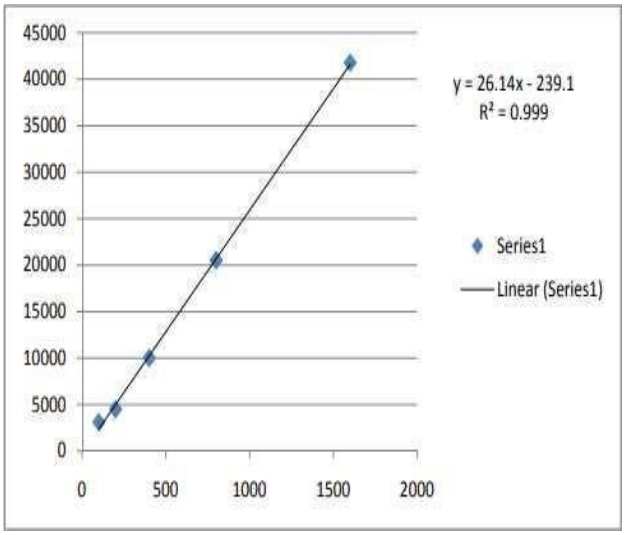


Time	Height	Area	Area%
5.948	474624	41745.555	66.524
7.280	312340	21007.363	33.476

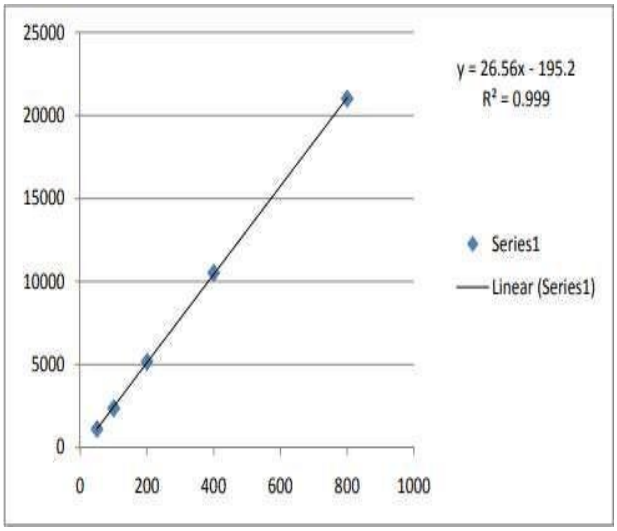
Chromatogram of Azelnidipine & Telmisartan (80/160µg/ml)

Linearity

The stock standard solution of Telmisartan and Azelnidipine was diluted appropriately with diluents to obtain standard solutions within the concentration range of 10-160 µg mL^{-1} of Telmisartan and 5-80 µg mL^{-1} of Azelnidipine. Each standard solution was injected three times into the UPLC system under the above-mentioned chromatographic working conditions. Linearity of the proposed method has been estimated at 5 concentration levels in the range 0f 10-160 and 5-80 µgmL^{-1} for Telmisartan and Azelnidipine by regression analysis. The calibration curve was developed by plotting average peak area versus standard concentration. (shown in fig 3 and 4).



Standard calibration graph of Telmisartan



Standard calibration graph of Azelnidipi

Specificity/selectivity

The chromatogram of Telmisartan and Azelnidipine standard solution has been given in (A). There is Two peak at the retention time of 5.950min and 7.293 min in this chromatogram. There are no other peaks caused by impurities in this chromatogram. There are no other peaks caused by contents of the mobile phase in this chromatogram(C). This indicates that the analytical method is specific.

Precision

Precision study was performed by injecting six times of standard solution at three different concentrations 20,40,80 µg mL^{-1} of Telmisartan and 10,20,40µg mL^{-1} of Azelnidipine on the same dayand three consecutive days. The precision data were given in Table 4. All RSD values for retention time and peak area for selected Telmisartan and Azelnidipine concentrations were less than 0.5 and 2.0%, respectively. In this case, the method is precise and can be used for our intended purpose. Table3.

SL.N	Sample Nam	Intraday Precision		Interday Precision	
		Conc(µg/ml	%RSD	Conc(µg /ml)	%RSD
01	Telmisartan	20	1.16	20	1.18
		40	1.52	40	1.51
		80	1.02	80	1.01
02	Azelnidipine	10	1.03	10	1.02
		20	1.14	20	1.13
		40	0.51	40	0.52

Accuracy study

A known quantity of standard solution has been added to the sample solutions previously analyzed at three different levels (50%, 100% and 150%). The amount recovered for Telmisartan and Azelnidipine has been calculated for three concentrations. The recovery data were summarized in (Table 4 and 5). Percent RSD values for all analyses were less than 2% indicating that excipients found in pharmaceutical formulations do not interfere and analytical method is very accurate.

Accuracy of Telmisartan

Sl. N	% of d added	Amount drug ta (µg/ml) (STD)	Amount drug ad (µg/ml) (sample)	Total am of drug(n=	Total amof drugfo	% Recovery	Mea	%S	%RS
1	50%	20	10	30	30	100	99.7	0.12	0.115
					29.9	99.2			
					30	100			
2	100%	20	20	40	40	100	99.4	0.67	0.77
					40.2	99.8			
					38.8	98.5			
3	150%	20	40	60	60	99.1	99	0.57	0.57
					59.9	98			
					58.9	100			

Accuracy of Azelnidipine

Sl. N	% of drug added	Amount of drug taken (µg/ml)(STD	Amount o drug add (µg/ml) (sample)	Total amount o drug(n=3	Total amount drugfou	% Recover	Mean	%SD	%RS
1	50%	40	20	60	59.9	99.8	99.9	0.94	0.94
					60.0	100			
					60.0	100			
2	100%	40	40	80	78.8	98.5	99.5	0.77	0.77
					80.2	100			
					80.0	100			
3	150%	40	80	120	100	100	99.6	0.47	0.47
					99.9	99			
					100	100			

Stability Studies: The stability study was investigated for the concentration sample under various stability periods and storage conditions. They were carried out at three concentration levels (low, medium and high) at six replicates. The percentage stability was estimated by comparing the mean of back calculated concentrations of all analytes from the stored stability samples with that of freshly samples (Table 6 and 7).

Stability Studies for Telmisartan.

Stability	sample Conc.(µg/ml)	Mean ± SD (µg/m	Recovery (%)	RSD (%
Stock solution hours.)	20	20.8±11.2	103.19	1.86
	40	40.8±12.3	102.40	2.15
	80	81.1±13.3	104.3	3.86
Bench top (1hours)	20	20.5±14.6	104.1	1.95
	40	40.8±23.1	105.5	4.9
	80	81.6±21.5	109.1	4.3
In injector (2hours)	20	21.5±14.2	105.0	3.0
	40	41.6±25.4	109.9	5.48
	80	81.3±30.2	108.9	6.2
Freeze-Tha	20	21.3±29.2	108.9	6.3
	40	41.6±17.5	107.4	3.7
	80	81.9±29.7	109.7	6.1

Stability Studies for Azelnidipine

Stability	Sample Conc. (µg/ml)	Mean ± SD (µg/ml)	Recovery (%)	RSD (%)
Stock solution (hours)	10	10.8±12.2	104.19	2.67
	20	21.8±15.3	106.40	3.26
	40	40.1±13.3	105.3	2.86
Bench top (1hours)	10	10.5±14.6	105.1	1.9
	20	21.8±23.1	107.5	4.9
	40	40.1±21.5	109.1	4.3
In injector (hours)	10	10.5±14.2	105.0	3.0
	20	20.6±25.4	109.9	5.48
	40	40.3±30.2	108.3	6.2
Freeze-Tha	10	10.3±29.2	108.9	6.3
	20	20.6±17.5	107.4	3.7
	40	80±29.7	109.7	6.1

CONCLUSIONS

A very quick, cost-effective, precise and accurate UPLC method for the determination of simultaneous estimation for Telmisartan and Azelnidipine has been developed and validated in compliance with ICH guidance Q2. Besides the short run time (10 min), retention time For Telmisartan and Azelnidipine was found to be 5.950 and 7.293 minutes respectively and flow rate of mobile phase ($0.8mLmin^{-1}$) made the method attractive because these features save analysis time and cost. In short, this method is, selective, reproducible and rapid for Telmisartan and Azelnidipine in API. The accuracy and precision are within reasonable limits and finally Analytical method is reliable, Precise And Accrued.

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