IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Development And Validation Of RP-HPLC Method For Simultaneous Estimation Of Telmisartan And Azelnidipine In Their Combined Marketed Formulation

Mayur Anant Jawanjal, Lokesh R. Gandhi, Nitin. S. Bhajipale

[1]Student, [2]Associate Professor, [3]Principal

[1]M. Pharmacy in Quality Assurance,

[1]SGSPS Institute of Pharmacy, Akola, India.

Abstract: A Simple, rapid, economical, precise and accurate Reverse Phase Method High Performance Liquid Chromatography (RP – HPLC) Method for Simultaneous Estimation of Telmisartan and Azelnidipine in their combined dosage form has been developed. The RP- HPLC method was developed for the simultaneous estimation of Telmisartan and Azelnidipine in their combined dosage form development method has been achieved. The separation was attained by Column – C8 150 x 4.6 mm, Zodiac. ID. 5µ particle size and 0.1% Formic Acid buffer Acetonitrile (50:50 v/v)as mobile phase at a Flow rate 1 ml per minute , Detection was carried out of wavelength of 257 nm retention time of Telmisartan and Azelnidipine were found to be 6.16 min and 8.94 min respectively. The method has been validated for linearity, accuracy, robustness and precision. Linearity observed for Telmisartan 62.5-3.9 µg/ml and for Azelnidipine 62.5-3.9 µg/ml. The percentage recovery obtained for Telmisartan and Azelnidipine were found to be in range 100.15 ± 0.50 and 100.53 ±70 respectively. Development method was found to be accurate, precise and rapid for simultaneous estimation of Telmisartan and Azelnidipine in their combined dosage form.

Index Terms - **RP – HPLC, Zodiac and Formic acid buffer, Acetonitrile, Telmisartan, Azelnidipine.**

1.INTRODUCTION

1.1 Introduction to Diseases

Hypertension is the most common cardiovascular disease. As many as 50 million people in the United States have systolic and/or diastolic blood pressure above 140/90 mmHg. Elevated arterial pressure causes pathological changesin the vasculature and hypertrophy of the left ventricle. Asa consequence, hypertension is the principal cause of stroke, leads to disease of the coronary arteries with myocardial infarction and sudden cardiac death, and is a major contributor to cardiac failure, renal insufficiency, and dissecting aneurysm of the aortae. However, from the standpoint of health promotion, it should be noted that the risk of both fatal and nonfatal cardiovascular disease in adults is lowest with systolic blood pressures of less than 120 mm Hg and diastolic less than 80 mm Hg; these risks increase progressively with higher levels of both systolic and diastolic blood pressure.

1.2 Introduction to Analytical Method

Pharmaceutical products formulated with more than one drug, typically referred to as combination products. These combination products can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods. The development and validation of analytical methods [Spectrophotometric, High performance liquid chromatography (HPLC) & High performance thin layer chromatography (HPTLC)] for drug products containing more than one active ingredient. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products.

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing ones. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs.

1.2.1 Introduction to HPLC Method

Liquid chromatography (LC) is a physical separation technique conducted in the liquid phase. A sample is separated into its constituent components (or analytes) by distributing between the mobile phase (a flowing liquid) and a stationary phase (sorbents packed inside a column). For example, the flowing liquid can be an organic solvent forced through the column at high speed and the stationary phase can be porous silica particles packed in a column. The modern form of column chromatography has been called high performance, high Pressure, high-resolution and high-speed liquid chromatography. High-performance liquid chromatography (HPLC), sometimes called high-pressure liquid chromatography, is a separation technique based on a solid stationary phase and a liquid mobile phase.

There are different modes of separation in HPLC:

a. Normal phase mode.
b. Reversed phase mode.
c. Ion exchange chromatography.
d. Reverse phase ion pair chromatography.
e. Affinity chromatography and
f. Size exclusion chromatography

Parameters that are affected by the changes in chromatographic conditions:

a. Resolution (Rs).
b. Capacity factor (k').
c. Selectivity (α).
d. Column efficiency (N).
e. Peak asymmetry factor (As).

1.3 Drug Profile

1.3.1 Azelnidipine

IUPAC Name – 3-1-Benzhydryl-3-azetidinyl 5-isopropyl 2-amino-6-methyl-4-(m-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Chemical Profile –

Parameters	Azelnidipine
Molecular weight	582.646 g/mol
Molecular formula	C33H34N4O6
CAS No	123524-52-7
Melting point	122 - 123°C

Pharmacokinetic Parameters –

Parameters	Azelnidipine
Absorption	Orally absorbed
Metabolism	Metabolized by cytochrome P450 (CYP) 3A4 in the liver and has no active metabolite
Bioavailability	Less than 50%
Half life	16 - 24 hrs
C_{max}	3.0 - 13.1 mg/ml
Plasma protein binding	≈ 90%

Mechanism of Action –

Azelnidipine is Ca+2 channel blocker inhibits trans membrane Ca+2 influx through the voltage dependent channels of smooth muscle in vascular walls. Ca+2 channels are classified into various categories including L-type, T-type, N-type, P/Q- type, R-type Ca+2 channels. Normally, calcium induces smooth muscle contraction, contributing to hypertension. When calcium channels are blocked, the vascular smooth muscle does not contract, resulting in relaxation of vascular smooth muscle walls and decreased BP.

1.3.2 TELMISARTAN

IUPAC Name - 2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3benzodiazol-1-yl] methyl} phenyl) benzoic acid.

Chemical Profile –

Parameters	Telmisartan
Molecular weight	514.62 g/mol
Molecular formula	$C_{33}H_{30}N_4O_2$
CAS No	144701-48-4
Melting point	261-263°C

Pharmacokinetic Parameters –

Parameters	Azelnidipine
Absorption	Orally absorbed
Metabolism	Minimal Liver (glucuronidation)
Bioavailability	42-100%
Half life	24 hrs
Plasma protein binding	> 99.5% mainly to albumin and alpha-1- acid glycoprotein

Mechanism of Action –

Telmisartan interferes with the binding angiotensin II to the angiotensin AT_1 –receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland. A angiotensin II is a vasoconstrictor, which also stimulates the synthesis and release of aldosterone ,blockage of its effects results in decreases in systemic vascular resistance.

Telmisartan acts as a selective modulator of peroxisome proliferator activated receptor-γ, a regulator of insulin and glucose metabolism. It is believed that Telmisartan dual mode of action may provide protective benefits against the vascular and renal damage caused by diabetic and cardiovascular disease.

Details of Marketed Formulation –

Brand Name	Mfg. by	Content	Quantity(mg)
Telma-AZ	Glenmark Pharmaceuticals Ltd. Mumbai, India.	Azelnidipine	8
		Telmisartan	40

2. EXPERIMENTATION

2.1 APPARATUS AND INSTRUMENTS:

Sr No	Instrument	Make
1	HPLC System	Shimadzu (SCL-10AVP)
2	UV-Spectrometer	Shimadzu (1800)
3	Analytical Balance	Metler Toledo
4	Sonicator	Analab

2.2 Reagents and Chemicals:

Sr No	Name of Chemical	Manufacturer
1	Formic acid	Merck Ltd., India
2	Methanol	Merck Ltd., India
3	Acetonitrile	Merck Ltd., India
4	HPLC Water (AR grade)	Merck Ltd., India

2.3 Chromatographic Conditions:

Sr No	Parameter	Condition
1	Mobile phase	Methanol-Acetonitrile (0.1% Formic Acid) (50:50 v/v)
2	Wavelength	257 nm
3	Flow Rate	1.0 ml/min
4	Temperature	28˚C
5	Run Time	15.0 min

2.4 Preparation of Solutions

2.4.1 Standard Stock Solutions

Standard stock solutions of AZEL and TEL (1 mg mL-1) were prepared separately by dissolving 10 mg of the drug in methanol using a 20 mL volumetric flask and completing the final volume adjusted with methanol based on the solubility of drugs in particular eluents. Furthermore, freshly prepared sample solution was sonicated for 10 minutes and later filtered through 0.20µ nylon filters. Required serial dilution was made for evaluating the validation studies.

2.4.2 Working Standard Stock Solutions

Working stock solution of TEL (125 µg mL-1) was prepared by serial dilution of 1.25 ml of its stock solution in a 10 mL volumetric flask by completing to volume with the mobile phase. Working solution of ACP (125 µg mL-1) was prepared by serial dilution of 1.25 ml of its stock solution in a 10 mL volumetric flask by completing to volume with the mobile phase.

2.4.3 Marketed Sample Preparation

Exactly 20 tablets of Telma-AZ containing 8 mg of AZEL and 40 mg of TEL were weighed separately, powdered and mixed in a mortar. An accurately weighed 10 mg amount of the finely powdered Telma-AZ tablets were transferred into 100 mL volumetric flask and the volume was adjusted with 10 mL of acetonitrile and water and sonicated until completely dissolved. The solutions were filtered with 0.2 µ nylon filters, followed by serial dilutions to the required concentrations using the same mobile phase for experiment with standard addition technique.

3. OBSERVATIONS

3.1 Method Validation:

Parameters	Telmisartan	Azelnidipine
Theoretical plates (*N*)	11523	32784
Capacity Factor (*K'*)	2.10	3.49
Separation factor (α)	9.53	1.66
Tailing factor (*T*)	1.14	1.14

- **LOD AND LOQ**

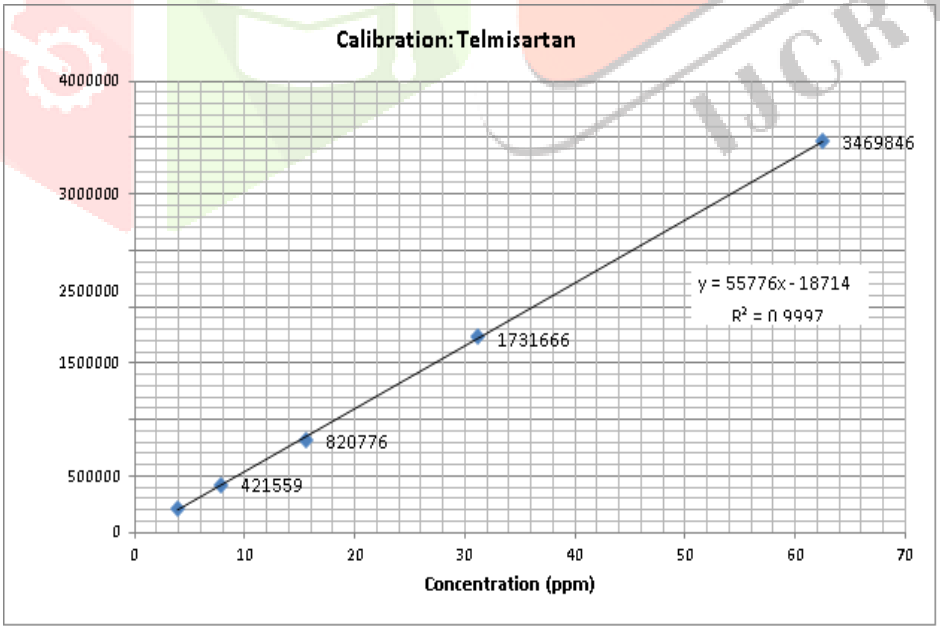
Parameters	Telmisartan	Azelnidipine
LOD	1.74 µg/ml	0.38 µg/ml
LOQ	5.82 µg/ml	2.33 µg/ml

3.1.1 Linearity and Range

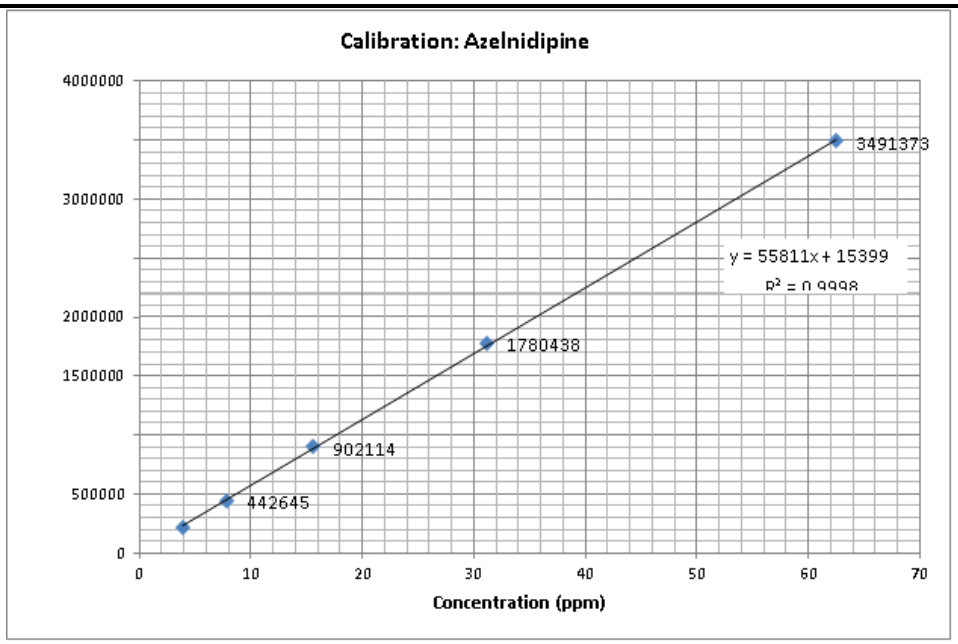
The linearity for Telmisartan and Azelnidipine were assessed by analysis of combined standard solution in range of 62.5-3.9 µg/ml and 62.5-3.9 µg/ml respectively. Correlation co- efficient for calibration curve Telmisartan and Azelnidipine was found to be 0.9997 and 0.9999 respectively.

The regression line equation for Telmisartan and Azelnidipine are as following:

For Telmisartan : y = 55776x – 39633 and For Azelnidipine : y = 55811x + 82885



Calibration curve of Telmisartan (62.5-3.9 µg/ml)



Calibration curve of Azelnidipine (62.5-3.9 µg/ml)

3.1.2 Precision

I.Repeatability

The data for repeatability of peak area measurement for Telmisartan (125 µg/ml) and Azelnidipine (125 µg/ml), based on six measurements of same solution of Telmisartan (125 µg/ml) and Azelnidipine (125 µg/ml). The % RSD for Telmisartan and Azelnidipine were found to be 1.18 and 1.13 respectively.

Drug	Concentration (µg/ml)	%RSD
Telmsartan	125	1.18
Azelnidipine	125	1.13

II.Intraday precision

The % R.S.D. for Intraday precision was found to be 0.69-1.30 for Telmisartan and 0.16 -1.34 for Azelnidipine.

III.Inter day precision

The % R.S.D. for inter day precision was found to be 0.44-1.91 for Telmisartan and 0.16 -1.34 for Azelnidipine.

Drug	Concentration (µg/ml)	%RSD	
		Intraday	Interday
Telmisartan	125	0.72	0.69
Telmisartan	125	1.30	1.91
Telmisartan	125	0.69	0.41
Azelnidipine	125	1.34	0.16
Azelnidipine	125	0.41	1.34
Azelnidipine	125	0.61	0.60

3.1.3 Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for Telmisartan was 99.93-100.65% and for Azelnidipine it was found to be in range of 100.53-101.18%.

Conc. (%)	Ref. Std. (mg)	Mkt. Drug (mg)	Recovery (mg)	% recovery	Mean Recovery (%)
80	40	32	72.1	100.14	100.15
	40	32	72.12	100.17	
	40	32	72.11	100.15	
100	40	40	81.25	101.56	100.65
	40	40	80.15	100.19	
	40	40	80.17	100.19	
120	40	48	88.17	100.19	99.93
	40	48	88.24	100.27	
	40	48	87.43	99.35	

Accuracy Data for Telmisartan

Conc. (%)	Ref. Std. (mg)	Mkt. Drug (mg)	Recovery (mg)	% recovery	Mean Recovery (%)
80	8	6	14.1	100.71	100.78
	8	6	14.12	100.86	
	8	6	14.11	100.79	
100	8	8	16.25	101.56	101.18
	8	8	16.15	100.94	
	8	8	16.17	101.06	
120	8	10	18.17	100.94	100.53
	8	10	18.24	101.33	
	8	10	17.88	99.33	

Accuracy Data for Azelnidipine

4. CONCLUSION

- ✓ A simple, specific, accurate and precise RP- HPLC method has been developed and validated as per ICH guideline for simultaneous estimation of Telmisartan and Azelnidipine in t heir combined dosage form.
- ✓ Validation parameters like Linearity, Accuracy, Precision, Robustness, System suitability,Specificity were tested.
- ✓ Observation of all these parameters leads to the point that developed RP-HPLC method is linear, accurate, precise, specific and robust.
- ✓ It can be successfully adopted for routine quality control analysis of Telmisartan and Azelnidipine in Combined dosage form without any interference from common excipients and impurity.
- ✓ This method can now transfer to utilize for routine laboratory analysis and assay of Telmisartan and Azelnidipine in their combined dosage form.

5. REFERENCES

1) FDA, "Guidance for Industry; Analytical Procedures and Methods Validation (Draft guidance), Food & Drug Administration," Rockville, US Department of Health and Human Services, 2000.
2) ICH, Validation of Analytical Procedures; Methodology, Q2 (R1), International Conference on Harmonization, IFPMA, Geneva 1996.
3) Indian Pharmacopoeia Commission Ghaziabad Vol.II, 2010, pp 1076-1077.
4) Kokilambigai KS, Lakshmi KS, Kumar A, Govind C, Kumar S, Singh MK, "Spectrophotometric Estimation Of Cilnidipine In Bulk And Pharmaceutical Dosage Form Using N-(1- Naphthyl) Ethylene Diamine Dihydrochloride. " Int. J. of Pharma and Pharma Sci.2014, 6, 576- 578.
5) Rao SN, Kumar AA, Bhimavarapu RD, Prasanna KV, "Estimation of Irbesartan in Bulk and Dosage Forms by New Simple UV-Spectrophotometry Using Hydrotropic Technique" Pharm. Anal. Acta., 2013, 4(8), 1-3.
6) Soni IJ, Panchal HJ,"Development and Validation of Dual Wavelength UV Spectrophotometric Method for simultaneous estimation of Cilnidipine and Olmesartan Medoxomil in Tablet dosage form." Ind. J. Pharma. and Bio. Res. 2014, 2, 76-81.
7) Mohammad SM, Yagaina NM, "Development and validation of a Rapid Stability Indicating chromatographic determination of Cilnidipine in Bulk and Dosage form" Ind. J. pharm. and Tech, 2013, 6(3), 296-299.
8) Zhang X, Zhai S, Zhao R, Ouyang J, Li X, Baeyens WR, "Determination of cilnidipine, a new calcium antagonist, in human plasma using high performance liquid chromatography with tandem mass spectrometric detection." Anal Chim Acta. 2007 , 1,142-146.
9) Kyeong-Ryoon Lee, Yoon-Jee Chae, Jong-Hwa Lee, Dae-Duk Kim, Saeho Chong, Chang-Koo Shim, and Suk-Jae Chung, "Quantification of Cilnidipine in human plasma by liquid Chromatography-mass spectrometry."J of Liq Chromato & Related Tech. 2012,35, 308–20
10) AzimMdSabir et al. A Review on High Performance Liquid Chromatography
11) Devolopment and Validation, Int. Res. J. Pharm. 2013; 4(4):39-46.
12) B. Lakshmi, K. Rama Krishna, K. N. Jayaveera and G.V.Padmakar Rao, New RP-HPLC method development and validation for the estimation of Iloperidone in Bulk drug and its pharmaceutical dosage forms, Rasayan Journal of Chemistry, Vol. 8 , No.1 , 2015, 59-66