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Method development and validation of telmisartan and amlodipine besylate by RP-HPLC in Tablet Dosage form

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Abstract

A simple, precise, accurate and rapid reverse phase high performance liquid chromatographic method had been developed for simultaneous estimation of Telmisartan and Amlodipine Besylate in tablet dosage form. SymmetryC18 4.6 x 250mm, 5m particle size was used. The method was carried out in gradient program using mobile phase, 0.02M Potassium dihydrogen orthophosphate: acetonitrile (30:70 v/v) adjusted to pH-5 using dilute ortho phosphoric acid. Flow rate was adjusted to 1.0ml/min and effluents were monitored at 245nm. The retention time obtained for Amlodipine Besylate and Telmisartan was 2.325 and 3.523 min respectively. The calibration curves were linear in the concentration range of 32-96µg/ml for Telmisartan and 4-12µg/ml for Amlodipine. The developed method was validated in accordance to ICH guidelines.

Keywords: Telmisartan, Amlodipine besylate, Acetonitrile, RP-HPLC

Contents

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1. Introduction

Telmisartan (1) is: 2-(4-{[4-methyl-6-(1-methyl-1 *H*-1,3-benzodiazol-2-yl)-2-propyl-1*H*-1, 3-benzodiazol-1-yl] methyl} phenyl) benzoic Acid. An angiotensin II receptor antagonist (ARB) used in the management of hypertension.

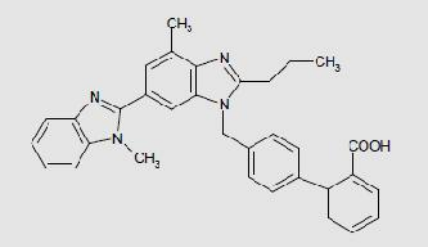

Fig.1 Structure for Telmisartan

Generally, angiotensin II receptor blockers (ARBs) such as telmisartan bind to the angiotensin II type 1 (AT1) receptors with high affinity, causing inhibition of the action of angiotensin II on vascular smooth muscle, ultimately leading to are duction in arterial blood pressure. Recent studies suggest that telmisartan may also have PPAR-gamma agonistic properties that could potentially confer beneficial metabolic effects. Absolute bioavailability depends on dosage. Food slightly decreases the bioavailability (a decrease of about 6% is seen when the 40-mg dose is administered with food)[1-8].

Amlodipine (2) is: 3-ethyl 5-methyl 2-[(2- aminoethoxy)methyl]-4-(2 chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate a long-acting 1,4-dihydropyridine calcium channel blocker. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By inhibiting the influx of calcium in smooth muscle cells, amlodipine prevents calcium-dependent myocyte contraction and vasoconstriction. A second proposed mechanism for the drug's vasodilatory effects involves pH-dependent inhibition of calcium influx via inhibition of smooth muscle carbonic anhydrase. Some studies have shown that amlodipine also exerts inhibitory effects on voltage-gated N-type calcium channels. N-type calcium channels located in the central nervous system may be involved in nociceptive signaling and pain sensation. Amlodipine is used to treat hypertension and chronic stable angina. Amlodipine is slowly and almost completely absorbed from the gastrointestinal tract. Peak plasma concentrations are reached 6-12 hour following oral administration. Its estimated bioavailability is 64-90%. Absorption is not affected by food [9-15].

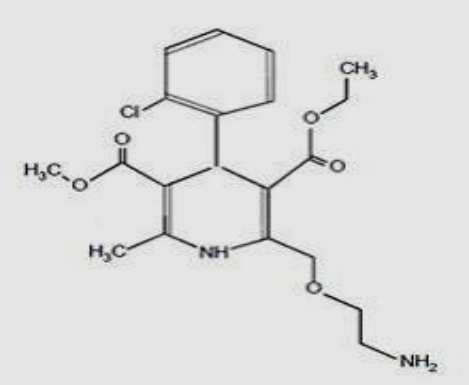

Fig.2 Structure for Amlodipine

2. Materials and Methods

Chemicals and solvents

Pure samples of TELMA and AMD were obtained respectively from sms pharmaceuticals labs, Hyderabad, India. The commercial pharmaceutical preparation containing 40mg and 5mg TELMA and AMD respectively (Marketed by Piramal Health care Pvt. Ltd) were procured from local pharmacy. Acetonitrile, Methanol and water used are of HPLC grade.

Instrumentation

The chromatographic separations were performed using HPLC-Shimadzu Model AX-200 consisting of an inbuilt auto sampler, a column oven and 2996 PDA detector. The data was acquired through Empower-2- software [16, 17]. Meltronics sonicator was used for enhancing dissolution of the compounds. Digisun pH meter was used for adjusting the pH of buffer solution. All weighing was done on sarotorious balance (model AX-200).

Chromatographic conditions

Column	:	Boston pH lex RP-C_{18} ODS (150 × 4.6 mm), 5µ
Mobile phase	:	Ammonium acetate (pH 3.0) buffer: ACN (70:30)
Pump mode	:	Isocratic
Flow rate	:	0.75 mL/min
Detection	:	236 nm.
Injection volume	:	20 µL
Column oven temperature:		Ambient
Run time	:	12 min

Preparation of Ammonium acetate buffer (0.01M): Accurately weighed 0.0770 g of ammonium acetate was transferred into 100 mL flask, 50 mL of double distilled water was added, sonicated and finally the volume was made up to mark with double distilled water. Then pH was adjusted to 3.0 with acetic acid .Filtered through whatman filter paper No. 40 [18].

Preparation of mobile phase: 70 mL of ammonium acetate buffer pH 3.0 (0.01M) and 30 mL of acetonitrile were mixed well and degassed in a sonicator for 5 min.

Preparation of standard and sample solutions of Amlodipine besilate and Telmisartan

Preparation of standard stock solution:

Accurately weighed 40.01 mg of telmisartan and 6.41 mg of amlodipine besilate (equivalent to 5 mg of amlodipine) were transferred into a 100 mL volumetric flask. 10 mL of methanol was added and sonicated to dissolve. 40 mL of diluent was added and sonicated for 10 min with intermittent shaking. Volume was made up to the mark with diluent and filtered through whatman filter paper No. 40.

Standard Solution:

5 mL of standard stock solution was pipetted out and transferred into a 50 mL volumetric flask. Volume was made up to the mark with diluent.

Preparation of sample stock solution:

20 tablets were weighed and the average weight of tablet was determined. The tablets were crushed into a fine powder. Accurately weighed and transferred 0.462 gm of powder equivalent to 5 mg of amlodipine into a 100 mL volumetric flask. 10 mL of methanol was added and sonicated for 15 min with intermittent shaking. 40 mL diluent was added and sonicated for 20 min with intermittent shaking. Volume was made up to the mark with diluent and filtered through whatman filter paper No. 40 [19, 20].

Sample Solution:

5 mL of standard stock solution was pipetted out and transferred into a 50 mL volumetric flask. Volume was made up to the mark with diluent.

Method Validation

The developed method was validated as per the ICH (International Conference on Harmonization) guidelines with respect to System suitability, Precision, Specificity, Linearity, Accuracy, Limit of detection and Limit of quantification.

Linearity:

The linearity was performed by preparing and injecting concentrations ranging from 20% to 140% (i.e., 20%, 40%, 60%, 80%, 100%, 120% and 140%) of the working concentration of amlodipine besilate and telmisartan covering seven points.

Calibration curve constructed by taking concentration on X-axis and area response on Y-axis, displayed good linearity over the concentration range. The polynomial regression for the calibration plot showed linear relationship with coefficient of correlation. Linearity range and correlation coefficient obtained for amlodipine and telmisartan were found to be 1-7 µg/mL, 1.000 and 8-56 µg/mL, 1.000 respectively

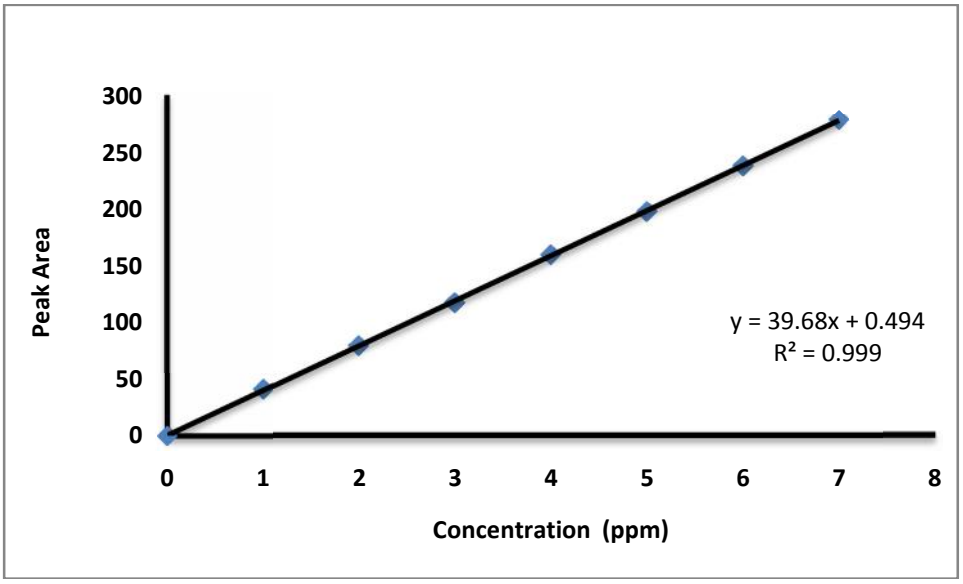

Fig.3 Linearity plot

Precision

System precision:

The system precision was performed by analyzing a standard solution of amlodipine besilate and telmisartan (6.3 µg/mL of amlodipine besilate and 40 µg/mL of telmisartan) for 5 times.

Table-1: System precision data for amlodipine and telmisartan

Injection No	Amlodipine		Telmisartan	
	Retention time (minutes)	Peak area	Retention time (minutes)	Peak area
1	2.22	197.957	6.7	3320.451
2	2.22	198.138	6.7	3329.210
3	2.22	197.001	6.74	3315.550
4	2.217	198.694	6.69	3274.742
5	2.22	197.643	6.7	3270.196
Mean	2.2194	197.886	6.723	3302.029
SD	0.001	0.55	0.03	24.71
% RSD	0.05	0.28	0.54	0.74

Acceptance Criteria: % RSD for the areas of five standard injections results should not be more than 2.0%
Method precision: Five assay samples of drug product at 100% of the working sample concentration were prepared and injected into the chromatographic system.

Accuracy: The accuracy was performed by spiking standard into 80%, 100% and 120% working concentration samples of amlodipine and telmisartan.
Standard stock solution: Accurately weighed 40.01 mg of telmisartan and 6.41 mg of amlodipine besilate (equivalent to 5mg of amlodipine) were transferred into a 100 mL volumetric flask. 10 mL of methanol was added and sonicated to dissolve. 40 mL of diluent was added and sonicated for 10 min with intermittent shaking. Volume was made up to the mark with diluent and filtered through whatman filter paper No. 40.
Sample stock solution: 20 tablets were weighed and the average weight of tablet was determined. The tablets were crushed into a fine powder. Accurately weighed and transferred 0.462 gm of powder equivalent to 5 mg of amlodipine into a 100 mL volumetric flask. 10 mL of methanol was added and sonicated for 15 min with intermittent shaking. 40 mL diluent was added and sonicated for 20 min. Volume was made up to the mark with diluent and filtered through whatman filter paper No. 40.
Preparation of standard spiked 80% sample:0.5 mL of standard stock solution was spiked into 4 mL of sample stock solution present in a 50 mL volumetric flask and volume was made up to the mark with diluent.
Preparation of standard spiked 100% sample: 0.5 mL of standard stock solution was spiked into 5 mL of sample stock solution present in a 50 mL volumetric flask and volume was made up to the mark with diluent.
Preparation of standard spiked 120% sample: 0.5 mL of standard stock solution was spiked into 6 mL of sample stock solution present in a 50 mL volumetric flask and volume was made up to the mark with diluent.

Table-2: Accuracy data for amlodipine and telmisartan

Sample	Accuracy	Peak Area	% Recovery	Mean % Recovery	Overall Mean %Recovery
AMLODIPINE	80%	176.095	98.95	MEAN=99.93 S.D = 0.81 %RSD = 0.81	MEAN= 99.45 S.D = 0.34 %RSD = 0.34
	80%	178.021	99.90		
	80%	180.031	100.95		
	100%	215.742	99.20	MEAN=99.14 S.D = 0.18 %RSD = 0.18	
	100%	214.960	98.89		
	100%	215.721	99.34		
	120%	255.487	99.23	MEAN=99.28; S.D = 0.08 %RSD = 0.08	
	120%	254.479	99.21		
	120%	255.082	99.40		
TELMISARTAN	80%	3024.228	101.00	MEAN=100.81; S.D = 0.26 %RSD = 0.26	MEAN= 99.97 S.D = 0.72 %RSD = 0.72
	80%	3021.412	100.44		
	80%	3024.193	101.00		
	100%	3626.003	99.10	MEAN = 99.05; S.D = 0.13 %RSD = 0.13	
	100%	3628.034	99.10		
	100%	3630.205	99.20		
	120%	4331.117	101.10	MEAN=100.05; S.D = 0.04 %RSD = 0.04	
	120%	4324.969	100.00		
	120%	4326.969	100.05		

Acceptance Criteria: The % Recovery for each level should be not less than 98.0% and not more than102.0%.

Ruggedness

Five assay samples of drug product at 100% of the working sample concentration were prepared and injected into the chromatographic system by different analysts.

Table.3 Ruggedness data for amlodipine and telmisartan

S.No	Analyst -1 (set-1)		Analyst -2 (set-2)	
	Amlodipine Sample Area	Telmisartan Sample Area	Amlodipine Sample Area	Telmisartan Sample Area
1	197.984	3320.451	198.732	3314.972
2	198.899	3298.861	196.899	3322.432
3	198.734	3322.562	196.921	3356.641
4	197.931	3324.642	196.738	3325.732
5	197.867	3321.892	196.791	3364.523
Mean	198.462	3317.6816	197.221	3336.86
SD	0.44	9.5	0.78	19.83
%RSD	0.22	0.28	0.39	0.59

Table.4 Overall results of ruggedness

	Amlodipine	Telmisartan
Average	197.749	3327.270
SD	0.81	18.27
% RSD	0.41	0.54

Acceptance Criteria:

%RSD of areas of 5 sample injections in each set should be not more than 2.0

Overall %RSD of areas of 10 sample injections (set-1 and set-2) should be not more than 2.0.

Robustness:

The Robustness was performed at different flow rates and different wavelengths by using working standard solution of telmisartan and amlodipine besilate.

Table-5: Robustness data for effect of wave length variation

S.No	Wave length (nm)	Amlodipine			Telmisartan		
		RT (min)	Efficiency(th.pl)	Asymmetry	RT (min)	Efficiency(th.pl)	Asymmetry
1	Low	2.21	3162	1.0	6.82	5432	0.9
2	Actual	2.21	2250	0.9	6.72	4417	0.8
3	High	2.21	4653	0.9	6.72	6324	1.0

Acceptance Criteria for System Suitability:

1. RSD for the peak areas of responses of three replicate injections of the standard Solution is not more than 2.0%.
2. The number of theoretical plates (N) for the Amlodipine and Telmisartan peaks is NLT 2000.
3. The Tailing factor (T) for the Amlodipine and Telmisartan peaks is NMT 2.0

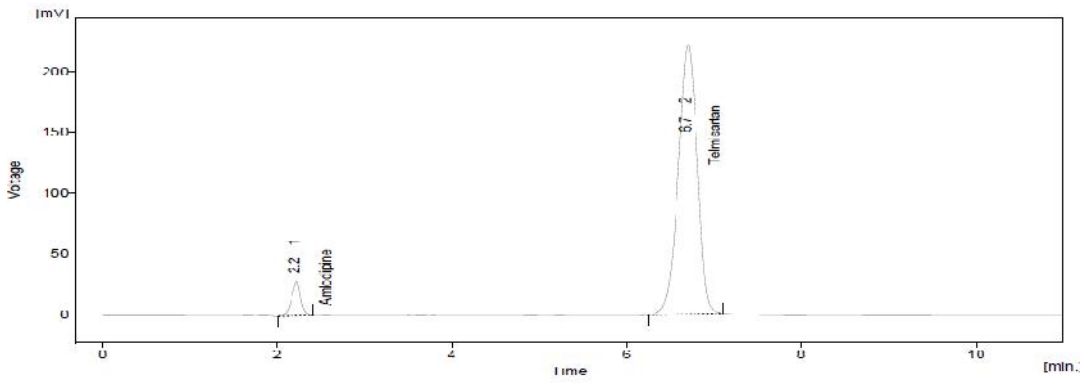

Table-6: Data of System Suitability (ASSAY)

S. No	Name	Retention time(min)	Area (mV.s)	Height (mV)	Resolution	Efficiency (th.pl)	Asymmetry
1	Telmisartan	6.72	3386.456	30.2	15.3	4417	0.8
2	Amlodipine besilate	2.21	198.646	227.4		2250	0.9

3. Conclusion

RP-HPLC method development: A simple, accurate, less expensive and more rapid reverse phase HPLC method was developed for the determination of amlodipine and telmisartan it was achieved by using Boston ph lex rp-c18 ods (150*4.6mm) column. The mobile phase used in this study was monobasic sodium phosphate at pH 2.5 adjusted with ortho phosphoric acid. The mobile phase was filtered through a 0.45µ membrane filter and degassed. The mobile phase was pumped from the solvent reservoir to column at a Flow rate of 0.75mL/min with injection volume of 20µL and the retention times was found to be 12.0 min and maximum absorbance was found to be 236 nm, this wavelength was selected for chromatographic method development. The assay results comply with label claim of the formulation. The developed method was validated as per ICH guidelines using parameters like accuracy, precision, linearity, specificity, ruggedness and robustness and found to be within the limits. In specificity no interferences was observed in test solution. The percentage recovery was found to be within the limits. In system precision and method precision RSD% was within limits. In ruggedness and robustness also the RSD% was within the limits.

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