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Research Article

Analytical Method Development and Validation and Force Degradation Studies for Simultaneous Estimation of Amlodipine Besylate and Telmisartan in Tablet Dosage Form by using RP-HPLC

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Abstract

The chromatographic analysis was performed on Athena C18 column(250×4.6mm, 5 µ particle size) with mobile phase consisting of methanol and phosphate buffer (pH 4) in the ratio of 70:30 v/v, at a flow rate of 1 mL/min and eluents monitored at 240 nm. The method was validated for linearity, accuracy, precision, robustness and application for assay as per International Conference on Harmonization (ICH) guidelines. The retention times of amlodipine besylate and telmisartan were 2.3 and 3.4 min, respectively. The calibration curves of peak area versus concentration, which was linear from 2.5-15 µg/mL for amlodipine besylate and 20-120 µg/mL for telmisartan, had regression coefficient (r2) greater than 0.998. The method had the requisite accuracy, precision, and robustness for simultaneous determination of amlodipine besylate and telmisartan in tablets. And force degradation also performed. The proposed method is simple, economical, accurate and precise, and could be successfully employed in routine quality control for the simultaneous analysis of amlodipine besylate and telmisartan in tablet form.

1. INTRODUCTION

Developed Countries in the world mainly suffered from the cardio vascular diseases like hypertension, peripheral artery disease, CHF and rheumatic heart diseases. Which diseases are main cause of millions of death cases. Till 2005 there are around 17.5 millions of death cases are due to Cardio Vascular Diseases. This amount will be representing around 30% of all the global deaths. According to calculations this will become almost 2o million by 2015.

High low density lipoprotein (LDL) cholesterol, raised blood pressure, increased serum homocysteine level and platelet aggregation are the main risk factors of this heart failure or cardio vascular diseases. Many chemical agents developed to treat cardio vascular diseases using drugs like Telmisartan, Amlodipine besylates etc Telmisartan[1] is chemically 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. Which is used in the treatment of hypertension and acts as an angiotensin II receptor antagonist. Telmisartan bind to Type 1 angiotensin II receptor and blocks the action of angiotensin II on vascular smooth muscles leads to reduced blood pressure.Recent proved that telmisartan also having PPAR-gamma agonistic properties that could potentially confer beneficial metabolic effects. A decrease of about 6% is seen when the 40-mg dose is administered with food. Amlodipine[2] is chemically 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2 chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate a long-acting1,4-dihydropyridine calcium channel blocker. It acts by stabilizing voltage-gated L-type Calcium channel, primarily on vascular smooth muscle. By preventing calcium-dependent myocyte contraction and vasoconstriction Amlodipine acts. pH-dependent inhibition of calcium influx via inhibition of smooth muscle carbonic anhydrase is another mechanism explained for Amlodipine. This cardi vascular disease drug also exhibit inhibitory effects on Voltage-gated N-Type calcium channel. Nociceptive signaling and pain sensation are the characters of the N-type calcium channel present in CNS. Amlodipine is a drug which is used in the treatment of hypertension and chronic stable angina. This drug was slowly and almost completely absorbed from the GIT. Around 6-12 hrs the peak plasma concentration were reached on oral administration. 64-90% bioavailability was estimated for amlodipine and moreover which was not affected by food. Present drug stability test guidance Q1A (R2) issued by international conference on harmonization (ICH)[3] suggest that stress studies should be carried out on a drug product to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability indicating and they should be fully validated. Accordingly, the aim of the present study was to establish inherent stability of Telmisartan, and Amlodipine besylate, through stress studies under a variety of ICH recommended test conditions[3-5] and to develop a rapid stability-indicating reverse phase high performance liquid chromatography assay method[6-8]. Literature survey reveals that a variety of spectrophotometric and chromatographic methods including UV, colorimetric determination, ratio derivative, and a stability-indicating HPLC methods have been reported for determination T A and H either single or in combination with other drugs[9-14]. Determination of telmisartan in human plasma by liquid chromatography-tandem mass spectrometry[15] , RP-HPLC method for determination of telmisartan in combination with hydrochlorothiazide[16-17], linear sweep polarographic method for determination of telmisartan[18] and HPLC method for amlodipine besylate in plasma with amperometric detection and a single step solid phase sample preparation [19-20], LC-MS method for determination of amlodipine besylate in human plasma [21] were reported in the literature. Literature survey also reveals several RP-HPLC methods for determination of amlodipine besylate in combination with atorvastatin calcium[22-24] andspectrophotometric method for estimation of amlodipine besylate in combination with atorvastatin calcium[25].

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Hence a rapid simple reproducible reverse-phase performance liquid chromatography method was developed for simultaneous quantitative determination of Telmisartan and Amlodipine in pharmaceutical dosage forms and performed forced degradation studies.

2. MATERIALS AND METHODS

Pure telmisartan (TEL) and amlodipine besylate (AMLO) used as working standards, were gifts from Chandra labs Ltd., India. Tablets containing 10 mg of AMLO and 80 mg of TEL (TELMA AM) were obtained from local pharmacy and used within their shelf life period. Methanol and water (HPLC-grade) were purchased from Merck, India. All other chemicals and reagents employed were of analytical grade, and purchased from Merck, India. Photassium dihydrogen phosphate and ortho phosphoric acid were analytical grade.

2.1 Buffer Preparation

1.625gm of photassium dihydrogen phosphate and 0.3gm diphotassium hydrogen phosphate is dissolved in 1000ml of water and the PH is adjusted with ortho phosphoric acid to 4.

2.2 Instrumentation

A Shimadzu HPLC system consisting of a, a column oven and dual wavelength absorbance detector (DAD) was employed throughout the analysis. The data were acquired through the spinchrone software. The column used was ODS Athena C18 (250×4.6 mm, 5µm). A Bandline sonerex sonicator was used for enhancing the dissolution of the compounds.

2.3 Optimized Chromatographic Conditions

The chromatography elution was carried out in the isocratic mode using a mobile phase consisting of acetonitrile and phosphate buffer (pH 4, pH adjusted with ortho phosphoric acid) in a ratio of 40:60 v/v. The analysis performed at ambient temperature using a flow rate of 1 mL/min with a run time of 10 min. The eluent was monitored using DAD at a wavelength of 240 nm. The mobile phase was filtered through whatmann filter paper No.41 prior to use.

2.4 Preparation of Stock and Standard Solutions

A stock solution of TEL and AMLO (1000 µg/mL) was prepared by taking accurately weighed 100 mg of TEL and AMLO reference standard in 100 mL volumetric flask containing 50 mL deionized water and then the volume was made up to the mark with deionized water. The stock solution is protected from light using aluminum foil. Aliquots of the standard stock solution of TEL and AMLO were transferred using A-grade bulb pipette into 100 mL volumetric flasks and solutions were made up to the mark with the mobile phase to give the final concentrations of 48-112 µg/ml and 6-14 µg/ml, respectively.

2.5 Estimation of Telmisartan and Amlodipine Besylate from Tablets

To determine the content of TEL and AMLO in tablets (Label claim: 40 mg and 5 mg), 20 tablets were taken and the contents were weighed and mixed. An aliquot of powder equivalent to the weight of one tablet was accurately weighed and transferred to 50 mL volumetric flask and was dissolved in 25 mL of deionized water and volume was made up to the mark with deionized water. The flask was sonicated for 25min to affect complete dissolution. The solution filtered through a 0.45 µm micro filter. A suitable aliquot of the filtered solution was transferred into a100 mL volumetric flask and made up to the volume with the mobile phase to yield the concentration of 50µg/mL for TEL and 8µg/mL for AMLO. The experiments were performed six times under the optimized chromatographic conditions described above. The peak areas were measured at 240 nm and concentration in the sample was determined by comparing the area of sample with that of the standard.

2.6 Method validation

2.6.1 Linearity

By appropriate aliquots of the standard TEL and AMLO solution with the mobile phase, five working solutions ranging between 48-112 µg/mL and 6-14 µg/mL were prepared. Each experiment was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of TEL and AMLO to obtain the calibration curve.

2.6.2 Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of TEL and AMLO to which known amounts of standard TEL and AMLO corresponding to 80,100 and 120% of label claim were added. The accuracy expressed as the percentage of analyte recovered by the proposed method.

2.6.3 Precision

Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines. The intra-day and inter-day precision were determined by analyzing the samples of TEL and AMLO at a concentration of 40, 60, 80µg/mL and 5, 7.5, 10 µg/mL respectively. Determinations were performed with three replicates on the same day as well as on three consequent days.

2.6.4 Limit of detection and the limit of quantification

Limit of detection (LOD) and limit of quantification (LOD) was calculated based on the ICH guidelines.

2.6.5 Robustness

The robustness of the method was performed by deliberately changing the chromatographic conditions. The organic strength and buffer pH were varied by ±2% and 0.2 units, respectively.

3. RESULTS AND DISCUSSIONS

A RP-HPLC method was proposed as a suitable method for the estimation of TEL and AMLO in the tablet dosage forms. The best chromatographic conditions were adequately selected. The selection of mobile phase and flow rate was made on the basis of peak shape, baseline drift, time required for analysis, and the mobile phase consisted of acetonitrile and phosphate buffer (pH 4, adjusted pH with ortho phosphoric acid) in the ratio of 40:60 v/v at a flow rate of 1mL/min and analyzed at 240 nm. The retention time observed (2.3 for AMLO and 3.4 for TEL) allows a rapid determination of these drugs. The typical representative chromatograms obtained under these conditions is shown fig.1-3.

The calibration plot (Fig.5 and 6) of peak area against concentration was linear in the range of 48-112 µg/mL and 6-14 µg/mL for TEL and AMLO respectively. The linear regression data for the calibration curves were indicative of a good linear relationship between peak area and concentration over a wide range. The correlation coefficient was indicative of high significance. The LOD and LOQ were determined based on analytical responses on 3 and 10 times the background noise, respectively.

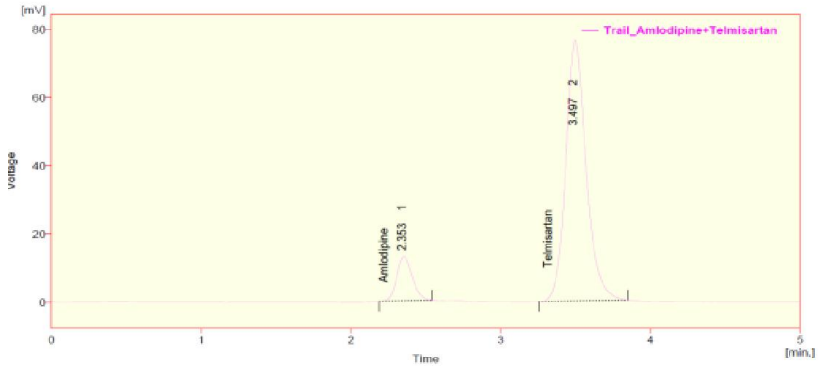
The accuracy was assessed from three replicates containing a concentration range of 80, 100 and 120%. The recovery of the method determined by spiking a previously analyzed test solution with standard TEL and AMLO solution, and the recovery values were found to be in the range of 101.26 and 101.40% respectively. The values of % recovery and %RSD were indicates that the method is accurate.

The precision of the method was assessed in accordance with ICH guidelines. The low %RSD (<2) values indicate that the method is precise. Reproducibility of the method was performed in the same laboratory on a same instrument which was performed by another analyst. The assay values and low %RSD (<2) values indicate that the method is reproducible.

The robustness was determined by analyzing the same sample under a variety of conditions. The factors consider being variations in the pH (0.2 units) and strength of acetonitrile (±2%).The results and the experimental range of the selected variables, together with the optimized conditions. There were no significant changes in the chromatography pattern when the above modifications were made in the experimental conditions, showing that the method is robust. The system suitability tests were also carried out to evaluate the reproducibility of the system for the analysis to be performed

The proposed method was applied to the analysis of marketed formulations. The blank solution was prepared containing the components indicated in tablet dosage form except the active ingredient. No interference was observed from the tablet excipients. In forced degradation studies there is no degradation occurred.

The results of method validation are summarized in Table no. 1 to 5.



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.353	92.914	12.943	11.37
2	3.497	724.182	76.538	88.63
	Total	817.095	89.481	100.00

Column Performance Table (From 50% - Trail_Amlodipine+Telmisartan)

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.353	0.113	1.286	2389	23887	-
2	3.497	0.140	1.351	3456	34559	5.311

Figure 1: A typical chromatogram of amlodipine and telmisartan

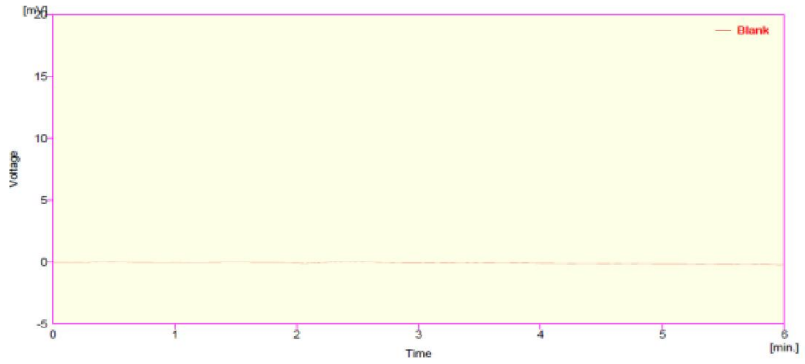
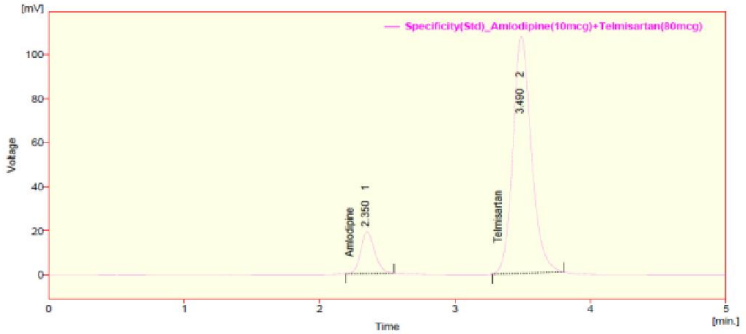


Fig. 2: Chromatogram of blank



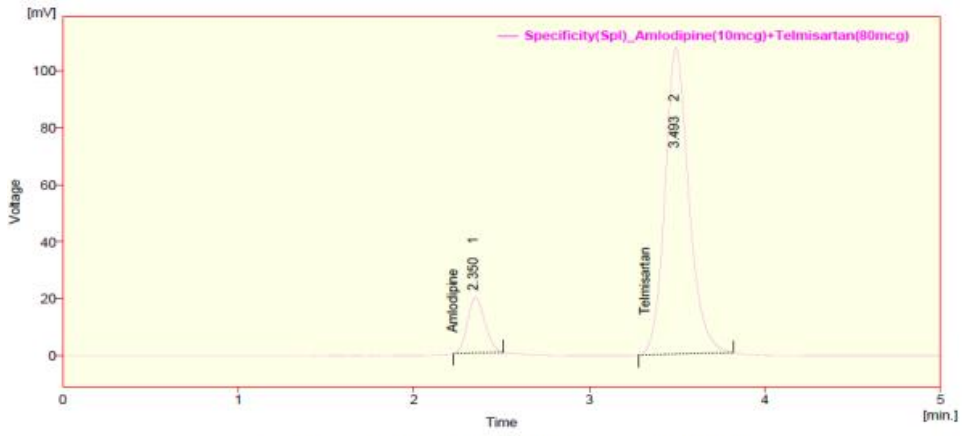
Specificity(Std)_Amlodipine(10mcg)+Telmisartan(80mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.350	135.898	18.973	11.75
2	3.490	1021.145	107.895	88.25
	Total	1157.044	126.869	100.00

Column Performance Table (From 50% - Specificity(Std)_Amlodipine(10mcg)+Telmisartan(80mcg))

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.350	0.113	1.370	2382	23819	-
2	3.490	0.143	1.289	3284	32845	5.227

Fig. 3: Chromatogram of standard.



Specificity(Spl)_Amlodipine(10mcg)+Telmisartan(80mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.350	135.146	19.538	11.70
2	3.493	1020.218	107.459	88.30
	Total	1155.363	126.996	100.00

Column Performance Table (From 50% - Specificity(Spl)_Amlodipine(10mcg)+Telmisartan(80mcg))

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.350	0.113	1.346	2382	23819	-
2	3.493	0.143	1.316	3291	32908	5.242

Fig. 4: Chromatogram of tablet sample

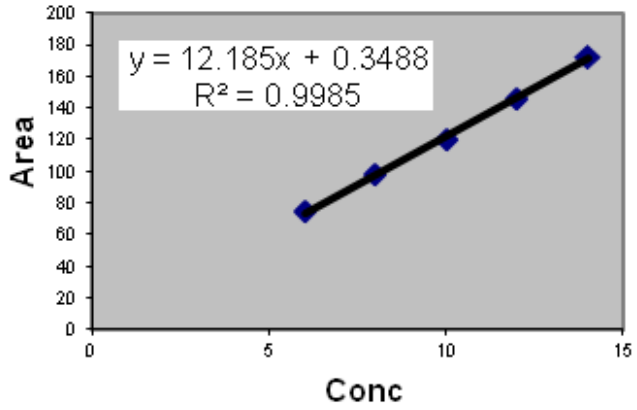


Fig. 5: Graph for linearity data of Amlodipine.

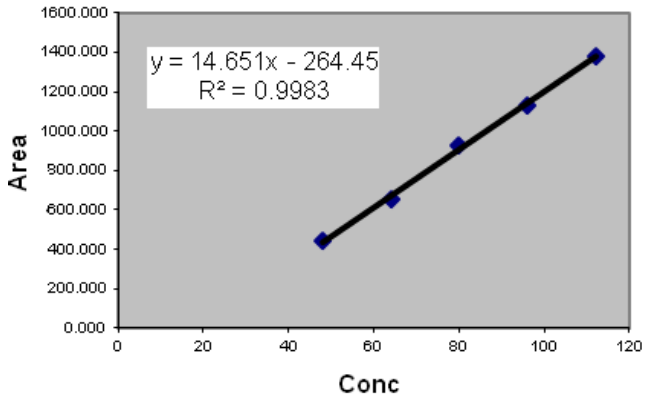


Fig 6: Graph for linearity data of Telmisartan

Table 1: Results of accuracy study

	Amlodipine	Telmisartan
1	140.97	1027.058
2	135.989	1015.619
3	135.114	1005.062
4	149.17	1023.142
5	135.898	1021.145
Average	137.358	1018.405
Sample area		
1	141.308	1023.211
2	135.217	1035.171
3	137.754	1029.375
4	135.217	1035.171
5	135.256	1035.333
Average	136.950	1031.652

Table 2: Calculation for assay of standard and sample Amlodipine and Telmisartan

Content	Amlodipine	Telmisartan
Tablet average weight	120.2mg	120.2mg
Standard weight	10mg	80mg
Sample weight	240.2mg	240.2mg
Label amount	5mg	40mg
Std. purity	99.64%	99.71%
Drug content	**4.97mg**	**40.44mg**
Assay	**99.43%**	**101.09%**

Table 3: Results of recovery study

Drug	Amlodepine	Telmisartan
Amount Added	10 mcg	80 mcg
	120.148	835.01
	125.519	826.185
	126.129	826.411
Average Area	123.932	829.202
Amount Recovered	10.13 mcg	81.12 mcg
% Recovery	101.26%	101.40%
Amount Added	12 mcg	96 mcg
	142.812	1101.576
	140.936	1091.906
	141.942	1102.198
Average Area	141.897	1098.560
Amount Recovered	11.81 mcg	94.66 mcg
% Recovery	98.43%	98.61%
Amount Added	14 mcg	112 mcg
	170.394	1325.968
	170.776	1315.295
	169.476	1326.671
Average Area	170.215	1322.645
Amount Recovered	14.01 mcg	112.21 mcg
% Recovery	100.06%	100.19%

Table 4: Results of precision study

S. No.	Amlodipine		Telmisartan	
	Rt	Area	Rt	Area
1	2.363	136.473	3.510	1015.834
2	2.35	135.444	3.500	1026.375
3	2.36	133.190	3.510	1023.904
4	2.363	133.075	3.513	1023.366
5	2.32	135.357	3.470	1041.225
6	2.357	133.526	3.503	1021.136
Avg.	2.3522	134.511	3.501	1025.307
Stdev	0.0165	1.429	0.016	8.572
% RSD	**0.70**	**1.06**	**0.46**	**0.84**

Table 5: Results of LOD and LOQ

	Amlodipine		Telmisartan	
	mcg	Area	mcg	Area
LOD	0.86	10.45	5.70	83.56
LOQ	2.60	31.66	17.27	253.22

4. CONCLUSION

The proposed RP-HPLC method is rapid, specific, accurate and precise for the quantification of TEL and AMLO from its tablet dosage form. The method has been found to be better than previously reported methods, because of its wide range of linearity, use of readily available mobile phase, lack of extraction procedures. All these factors make this method suitable for quantification of TEL and AMLO in tablet dosage forms. The method can be successfully used for routine analysis of TEL and AMLO in bulk drugs and pharmaceutical dosage forms without interference and forced degradation also performed but there is no degradation.

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