Development and Validation of a Reversed Phase HPLC Method for Simultaneous Determination of Amlodipine and Telmisartan in Pharmaceutical Dosage Form

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Abstract

A simple, accurate and precise method for the simultaneous determination of amlodipine and telmisartan in bulk drug and pharmaceutical dosage has been developed by RP-HPLC method. Separation was performed on a 5µm Nucleodur® C18 column (250 × 4.6mm ID) with acetonitrile: phosphate buffer at pH 4.5 (60:40v/v) in isocratic elution in less than 9 min with a flow rate of 1.3 ml min-1. Good sensitivity for all analytes was observed with UV detection at 238 nm. The method allowed quantification over the 1-11 µg ml-1 range for amlodipine and 8-80 µg ml-1 range for telmisartan. The method has been applied, without any interference from excipients or endogenous substances, for the simultaneous estimation of these two compounds in bulk drug and in tablets.

Keywords: Amlodipine, Telmisartan, Liquid chromatography.

Introduction

Telday® and Telma® tablets contain two nucleoside analogues namely amlodipine (AML) and telmisartan (TEL). Amlodipine and telmisartan belongs to the category of calcium antagonist, angiotension II antagonist respectively.

Telmisartan is an orally active nonpeptide angiotensin II antagonist that acts on the AT1 receptor subtype, effective agent for the treatment of hypertension and renal impairment. Chemically 2-[4-[[4-methyl-6-(1-methylbenzoimidazol-2yl)-2-propyl-benzoimidazol-1-yl] methyl] phenyl] benzoic acid. Angiotension II is the principal precursor agent of the rennin-angiotensin system [1], and its effects include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation, and renal reabsorption of sodium [2, 3]. It also prevents the glitazone-induced weight gain [5] without interfering with its insulin-sensitizing properties.

Amlodipine is a dihydropyridine calcium antagonist that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle [1]. Chemically 3-Ethyl 5-methyl

(4RS) -2-[(2-aminoethoxy)methyl] -4-(2-chlorophenyl) -6-methyl-1,4-dihydro pyridine -3,5-dicarboxylate benzene sulphonate. It binds to both dihydropyridine and non-dihydropyridine binding sites. Amlodipine is a peripheral vasodilator that acts directly on vascular smooth muscle to cause reduction in peripheral vascular resistance and reduction in blood pressure [2-4]. It also prevents the glitazone-induced weight gain without interfering with its insulin-sensitizing properties [5].

There have been several publications describing analytical methods for the determination of AML and TEL individually or with other drugs as combination. Most of the reported methods require solid phase extraction [6] or expensive equipment unsuitable for routine use in pharmacokinetic and pharmaceutical studies where many samples need to be analyzed. Amlodipine has been reported by, spectrophotometric method [7 - 10], HPLC [4, 8, 11-13], and electrospray ionization tandem mass spectroscopy [14, 15], LC-MS/MS [16], HPTLC [17] methods and telmisartan has been estimated by various methods like spectrophotometric [18], HPLC [19, 23], methods. However, there have been no reports concerning the simultaneous determination of AML, and TEL.

LC methods are widely used in the determination of drugs in pharmaceutical dosage forms [24-26]. The goal of this work was the development of a new, rapid, sensitive and fully validated method for the direct and simultaneous determination of AML, and TEL in raw materials, pharmaceutical dosage forms. This work aimed at the simultaneous determination of AML, and TEL.

Experimental

Equipment and Chromatographic Conditions

The chromatographic system consisted of Shimadzu make pump-LC-10ADvp with UV detector-SPD-10Avp. Separation was carried out with a Macherey Nagel make 5µm Nucleodur® C_{18} column (250×4.6mm ID), at 24ºC temperature. Isocratic elution was with a mixture of acetonitrile: phosphate buffer at pH 4.5 (60:40v/v) at a flow rate of 1.3 ml min-1. The UV detector was set at a wavelength of 238 nm. An injection volume of 20 µl was used.

Chemicals and Reagents

Amlodipine and Ttelmisartan in combination Telma® (claimed labeled amount 5mg AML and 40 mg TEL per tablet) was procured from local pharmacies. HPLC-grade acetonitrile and methanol were procured from Merck (Mumbai, India). All other chemicals (analytical grade) were obtained from Sigma (Bangalore, India) or Merck. Double-distilled water was used throughout.

Stock and Working solutions

Stock solution of amlodipine and telmisartan were prepared by weighing 35.67 mg of amlodipine besilate (eq. 25.62mg amlodipine) and 99.3 mg of telmisartan by dissolving separately in 50 % v/v aqueous methanol in 100 ml calibrated flask and made up the volume to get 256.2 µg ml^{-1} of amlodipine and 993 µg ml^{-1} of telmisartan in the final solutions. Further dilutions were made with 50 % v/v aqueous methanol to get 1.28µg $ml^{-1}$of AML and 9.93 µg ml-1of TEL considered as 100% test concentration. TEL was used as IS for AML and AML was used as IS for TEL.

Separate standard calibration graphs were constructed for each component by plotting the ratio of the peak area of the drug to that of IS against the drug concentration. The slopes, intercepts, correlation coefficient, and related validation parameters such as LOD, LOQ, standard error of slope and intercept were tabulated for each component.

All solutions were protected from light and were used within 24 h to avoid decomposition.

Ruggedness, Accuracy and Precision

The ruggedness and intra-day and inter-day precision and accuracy of the methods were estimated by assaying three replicate samples at three different concentrations, on the same day and on three different days over a two weeks period. For checking the ruggedness and precision of the method, the relative standard deviations (RSD) were calculated and tabulated. The accuracy of the methods was expressed as percentage bias [27, 28].Accuracy of the methods was also determined by recovery studies.

For Formulation

Twenty tablets, labeled as containing 5 mg of AML, and 40 mg of TEL together with excipients, were accurately weighed (8.69 g), and finely powdered. A weight of the powder equivalent to one tablet content was accurately weighed, transferred into a 100 ml calibrated flask, diluted with 80 ml of methanol and sonicated for 15 min for complete dissociation of the drug, and made up to the mark with methanol. The solution was filtered through Whatman filter paper 41 and the filtrate was collected in a clean flask. After filtration, 5 ml of the above solution was withdrawn and diluted to 50ml with mobile phase. The solution was filtered through a membrane filter (0.22µm) sonicated for degas.

Results and Discussion

This method provides a simple procedure to determine simultaneously the concentration of AML and TEL in bulk drugs and pharmaceutical dosage forms. To develop a rugged and suitable LC method, various mobile phase compositions, flow rate and different temperatures were tested (Table 1).

Table 1. Chromatographic conditions tried.

Parameter	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9
Mobile Phase	MeOH : Buffer (50:50)	ACN : Buffer (50:50)	ACN : Buffer (50:50)	ACN : Buffer (50:50)	ACN : Buffer (55:45)	ACN : Buffer (55:45)	ACN : Buffer (55:45)	ACN : Buffer (55:45)	ACN : Buffer (55:45)
Flow rate (ml/min)	1.5	1.5	1	1	1.1	1.2	1.2	1.2	1.3
pH	4.3	4.3	4.7	4.7	4.6	4.6	4.5	4.5	4.5
Temperature	24^0C	24^0C	24^0C	24^0C	24^0C	24^0C	24^0C	30^0C	24^0C
Wavelength	238 nm	238 nm	238 nm	238 nm	238 nm	238 nm	238 nm	238 nm	238 nm

MeOH = Meathanol, ACN = Acetonitrile

Preliminary trials using mobile phases consisting of a mixture of methanol, and acetonitrile ratios did not give good peak shape, analysis time, and leads to tailing factor. Addition of KH2PO4 buffer instead of methanol improved the peak shape of the compounds. Finally, by using a buffer pH 4.5 and a mobile phase consisting of a mixture of acetonitrile and buffer (5.5:4.5; v/v) at a flow rate of 1.3 ml min-1, AML and TEL, baseline resolution was obtained, and good peak shapes observed without tailing. After determining the optimum conditions, a satisfactory resolution was obtained in a short analysis time (Table 2). For all the compounds sharp and symmetrical well-resolved peaks were obtained.

Table 2. Optimized conditions for separation.

Parameter	Observations
Mobile Phase	Acetonitrile : Buffer (55:45)
Flow rate	1.3ml/min
Run time	10 min
Injection volume	20 µL
pH	4.5
Column	NUCLEODUR® 250 × 4.6 mm, RP-C_{18} of 5 µm particle size
Temperature	24^0C
Wavelength	238 nm

The USP suggests that system suitability tests to be performed prior to analysis [29]. The parameters include tailing factor, capacity factor, theoretical plate number, retention time, asymmetric factor, selectivity and RSD % of peak height or area for repetitive injections. Typically, at least two of these criteria are required to demonstrate system suitability for the proposed method. Some of tests were carried out on freshly prepared standard solutions. Tailing factors of 1.53, 1.22 were obtained for AML and TEL respectively, with asymmetry factors of 0.98 and 1.03. The theoretical plate number (N) was 2516 and 4888 for AML and TEL respectively. The chromatographic conditions described ensured adequate retention and resolution for both of the analytes. The retention times of AML and TEL were 3.49 and 8.40 min. the variation in retention time for five replicate injections of two compounds reference solutions gave RSDs of 0.314% for AML and 0.291% for TEL. the results obtained from the system suitability tests satisfy the USP requirements. The calibration curve and equations for AML and TEL in the mobile phase was calculated by plotting the peak area ratio of compound to IS vs concentration of compound in the range of 1-11 μg ml^{-1} and 8-80 μg ml^{-1} for AML (Figure 1) and TEL (Figure 2) in the mobile phase respectively. Linear regression parameters of the peak area ratio versus concentration of the compounds are presented in Table 3. These results showed highly reproducible calibration curves with correlation coefficients of > 0.999. The low SE values of the slope and the intercept in both media show

the precision of the proposed method. The LOD and LOQ were calculated from the following equations and using the standard deviation (s) of response and the slope (m) of the corresponding calibration curve.

LOD = 3.3 s/m; LOQ = 10 s/m [27, 28].

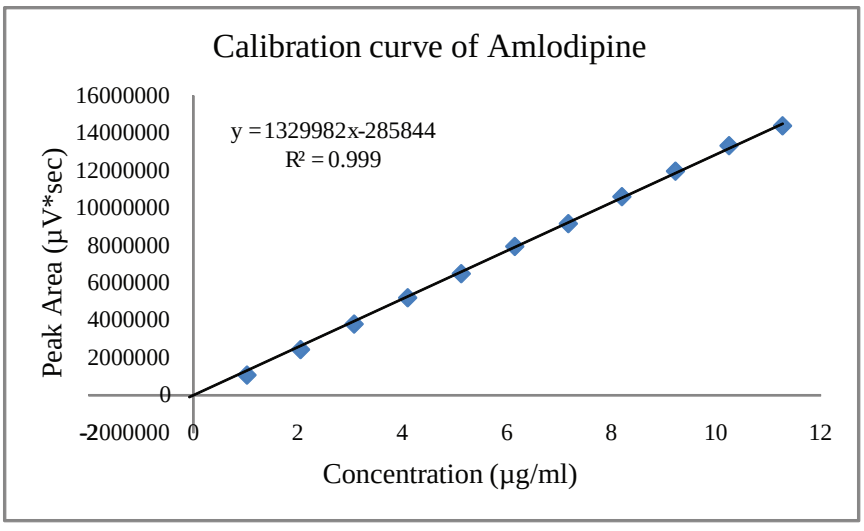

Figure 1. Linearity graph for AML.

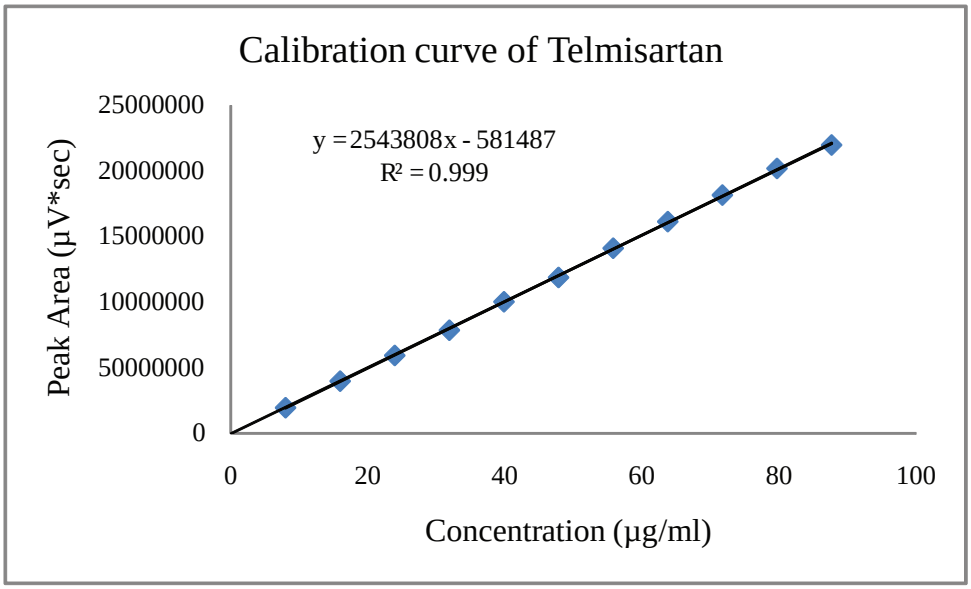

Figure 2. Linearity graph for TEL.

Precision, accuracy and reproducibility of the method were assessed by performing replicate analysis of standard solutions in the mobile phase. Repeatability and reproducibility were characterized by RSD% (Table 3). Based on these results, there was no significant difference for the assay, as tested by within-day (repeatability) and between-day (reproducibility).

Table 3. Statistical data for the calibration graphs of AML and TEL in mobile phase.

	AML	TEL
Linearity range (µg ml^{-1})	1 -11 µg ml^{-1}	8 -30 µg ml^{-1}
Slope	64.804	39.929
Intercept	2.4482	0.8612
Correlation coefficient	0.999	0.999
SE of slope	1.921×10^{-5}	1.024×10^{-5}
SE of intercept	2.732×10^{-2}	3.094×10^{-5}
LOD (µg/ml)	0.020668	3.3403
LOQ (µg/ml))	0.062629	10.122
Repeatability (RSD%)[a]	0.0516	0.1533
Reproducibility (RSD%)[b]	0.154	0.667

[a]Each value is obtained from five experiments.
[b]Between-day reproducibility is determined from five different runs over a 2 weeks period.

Recovery tests were carried out by analyzing synthetic mixtures of AML and TEL with different composition ratios (Table 4).

Table 4. Determination of AML and TEL in laboratory-made mixtures.

Added (μg ml^{-1})		Found (μg ml^{-1})		Recovery (%)	
AML (5)	TEL (40)	AML	TEL	AML	TEL
5	31.87	-	31.895	100.03	-
5	38.84	-	39.795	99.14	-
5	47.8	-	47.754	99.92	-
4.099	40	4.107	-	-	100.08
5.124	40	5.131	-	-	100.61
6.148	40	5.971	-	-	101.64
Mean recovery (%)				100.77	99.06
RSD (%)				0.7871	0.0580

[a]Each value is the mean of three experiments

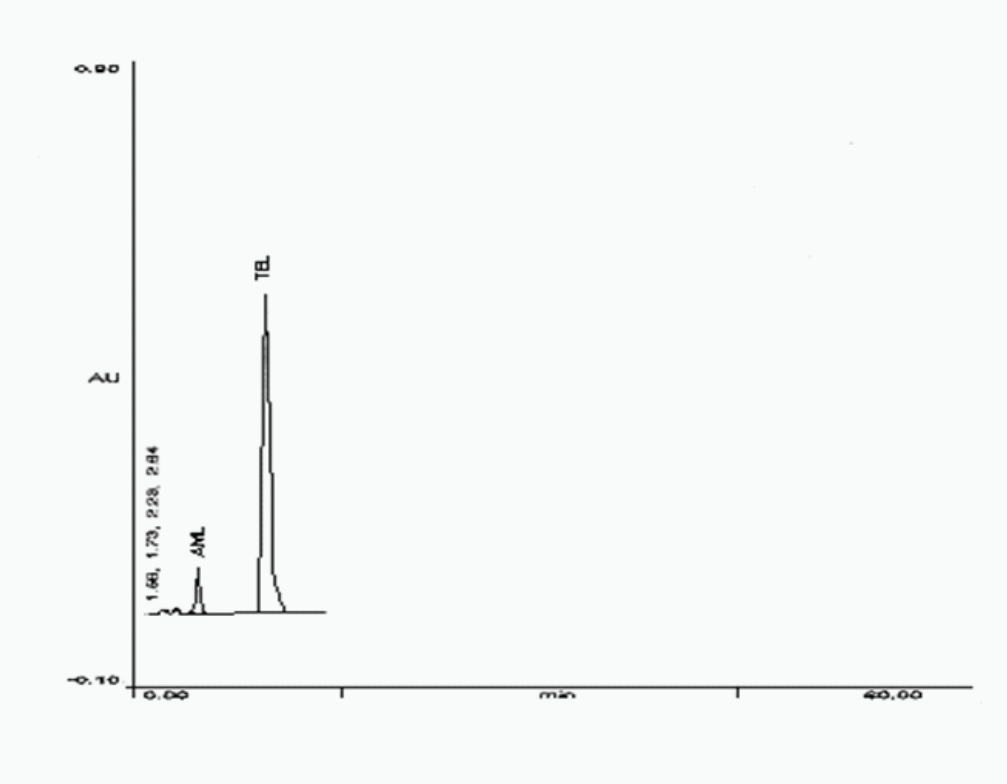

Figure 3. Chromatogram of AML, TEL.

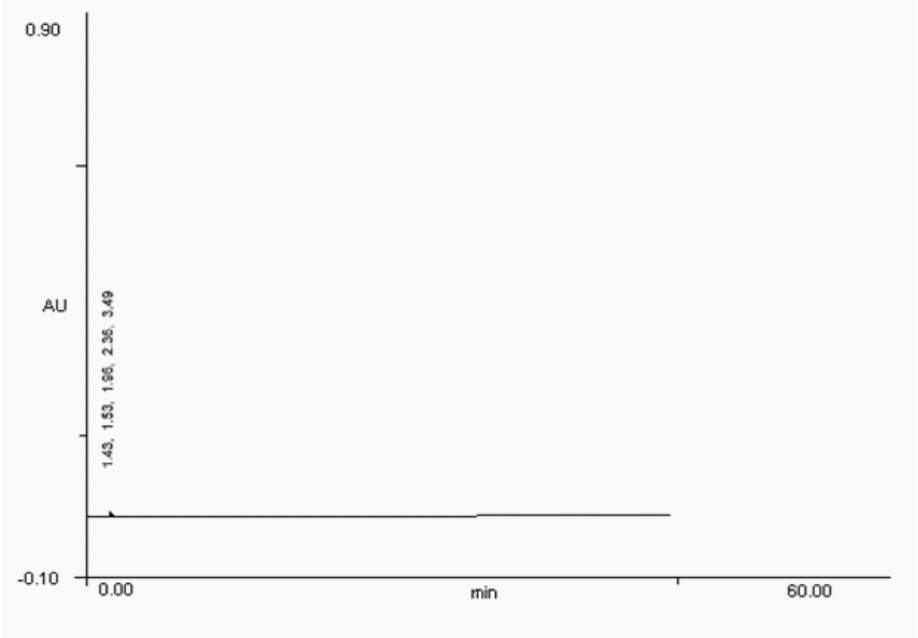

Figure 4. Representative Blank Chromatogram with excipients omitting AML, TEL.

Table 5. Results of the assay and the recovery analysis of AML, and TEL in tablet dosage forms.

	Tablets (mg)	
	AML	TEL
Labeled claim (mg per tablets)	5	40
Amount found (mg)[a]	5.031 µg/ml	40.248µg/ml
Added (mg)	4.09, 5.12, 6.14	31.87, 39.84, 47.8
Found[a]	9.03, 10.16, 11.0	72.14, 80.04, 88.0
Recovery (%)	100.08, 100.61, 100.64	100.03, 99.94, 99.92
RSD % of recovery	0.7871	0.0586

[a]Mean value of the five determination

Table 6. System suitability parameters.

S.No.	Parameter	Limit	Result
1	Resolution	R > 2	6.58
2	Injection precision	RSD <1% for n≥5	Amlodipine RSD = 0.0902% n = 6 Telmisartan RSD = 0.486% n = 6
3	Tailing factor	T ≤ 2	Amlodipine = 1.53 Telmisartan = 1.22
4	Theoretical plate	N > 2000	Amlodipine = 2516 Telmisartan = 4888
5	Retention time		Amlodipine = 3.49 min Telmisartan = 8.40 min

Recovery studies were also conducted with the tablets using the standard addition method to determine the accuracy and precision. The recovery was measured by spiking the already analyzed samples of tablets with known concentrations of standard solutions of the studied compounds. The results (Table 5) indicate the absence of interferences from the common pharmaceutical excipients used in the selected formulations. It is concluded that the method is sufficiently accurate and precise in order to be applied to the tablet dosage forms.

Conclusions

A RP-HPLC method has been developed for the simultaneous estimation of Telmisartan and Amlodipine in pharmaceutical dosage forms, using UV-detector. Different chromatographic conditions were used to develop the method. Elution was carried out with a mobile phase consisting of Acetonitrile: Buffer (55:45) at pH 4.5 ± 0.1, the flow rate was 1.3 ml min^{-1} at 238 nm. The retention time for Amlodipine and Ttelmisartan was found 3.48 and 8.42 minutes. Run time was found to be 10 minutes. The result of assay for Telma-AM tablet was found to be 98.71 ± 0.24%. Recovery of the Telmisartan and Amlodipine was found to be 99.75 ± 1.28%. It is evident from the study that the developed method is simple, specific, precise and accurate. The newly developed method can be used for routine analysis as method for the simultaneous estimation of Telmisatan and Amlodipine Besilate in pharmaceutical dosage forms.

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