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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF AMLODIPINE BESYLATE AND TELMISARTAN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, validated RP-HPLC method for the simultaneous estimation of Amlodipine Besylate and Telmisartan in pharmaceutical dosage form and bulk was developed for routine analysis. This method was developed by selecting Chromosil C18 (250×4.6mm column as stationary phase and acetonitrile: methanol (50: 50v/v) as mobile phase. Flow rate of mobile phase was maintained at 1 ml/min at ambient temperature throughout the experiment. Quantification was achieved with ultraviolet detection at 240 nm. The retention times of Amlodipine Besylate and Telmisartan were found as 5.2 min and 2.5 min respectively. The detector response was linear in the concentration range of 5- 80 µg/ml and 40-640 µg/ml of Amlodipine Besylate and Telmisartan respectively. The regression coefficient was 0.999 for both drugs. From recovery studies we concluded that the drugs have no interference with any excepients in the formulation. This method has been validated according to ICH guidelines and shown to be specific, sensitive, precise, accurate, rugged and robust. Hence, this method can be applied for routine quality control analysis of Amlodipine Besylate and Telmisartan in dosage form as well as in bulk drug.

KEYWORDS: Chromosil, Amlodipine Besylate and Telmisartan.

INTRODUCTION

Amlodipine Besylate[1] is a drug belongs to anti-hypertensive class used to treat hypertension

by blocking the calcium channels. The chemical structure Amlodipine Besylate is shown in Figure 1.

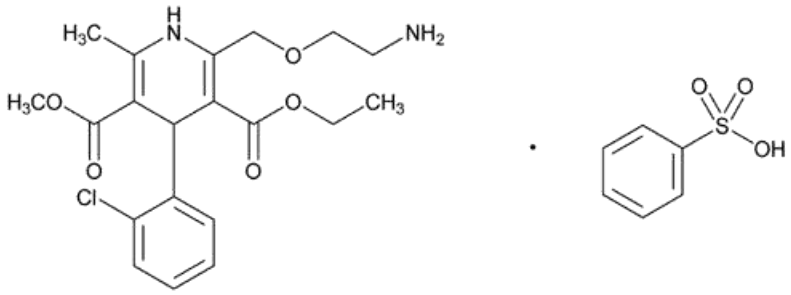

Figure 1: Chemical strutre of amlodipine besylate

The chemical name of Amlodipine Besylate is 2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl) -1, 4-dihydro-6-methyl-3, pyridine dicarboxylic acid 3-ethyl 5-methyl ester benzene sulphonate. The molecular formula of Amlodipine Besylate is $C_{20}H_{25}ClN_2O_5$. $C_6H_6O_3S$ and it has the molecular weight of 567.1 g/mol. It is slightly soluble in water and in isopropyl alcohol, sparingly soluble in dehydrated alcohol, freely soluble in methyl alcohol.

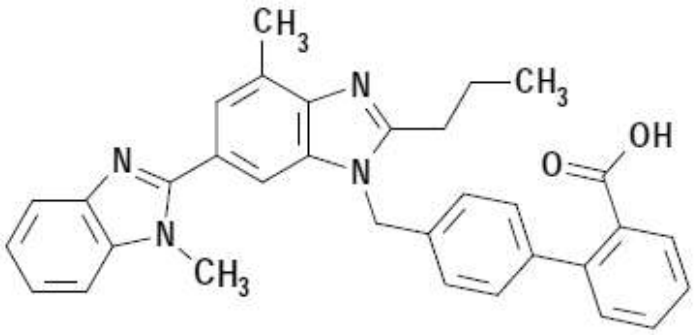

Figure 2: Chemical structure of Telmisartan

Telmisartan[2] is a drug belongs to anti-hypertensive class used to treat hypertension by blocking the angiotensin II receptors. The chemical structure Telmisartan is shown in Figure 2. The chemical name Telmisartan 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. The molecular formula is $C_{33}H_{30}N_4O_2$ and it has the molecular mass of 514.617 g/mol. It is soluble in methanol and poorly soluble in water. The two drugs which are mentioned above are official in Indian Pharmacopia[3] and United States Pharmacopoeia.[4] Literature review reveals various methods for determination of Telmisartan and Amlodipine besylate, individually and in combination with other drugs. A variety of analytical methods for estimation of Amlodipine are previously reported. The majority of methods reported are liquid chromatography coupled

to UV[5,6] fluorimetric[7], electrochemical[8,9], or mass spectrometry detection[10-13] but some determinations were also performed by thin layer[14,15], micellar electrokinetic[16] and gas chromatography[17,18] or spectrophotometry.[19,20] A LC method for the assay and related substances of Amlodipine besilate is also reported in the European Pharmacopoeia.[21] Due to their high sensitivity and selectivity, analytical methods such as liquid[22-26] or capillary gas chromatography were previously reported. Telmisartan in pharmaceutical dosage forms is determined by various techniquessuch as linear sweep polarography, parallel catalytic hydrogen wave method[27] and HPLC.[28-30] The statistical analysis proved that method is reproducible and selective for the simultaneous analysis of Amlodipine Besylate and Telmisartan in bulk and formulations.

MATERIALS AND METHODS

Gift samples of pure standard drugs Amlodipine Besylate and Telmisartan were supplied by Hetero Drugs Ltd., Hyderabad, India. The Methanol (HPLC grade), Acetonirile (HPLC Grade) were used. The commercially available tablets (one equivalent to 5mg Amlodipine Besylate and of 40mg of Telmisartan) which are manufactured by Cipla Ltd were purchased from market.

Instruments

HPLC system WATERS 2487 gradient system connected to UV detector and SHIMADZU AUY220 balance were used.

METHOD DEVELOPMENT

Chromatographic Conditions

Chromatographic separation was achieved by using Chromosil C18(250×4.6mm, 5µ) column as stationary phase and composition of Acetonitrile and Methanol (50: 50:v/v) as mobile phase. Flow rate was maintained at 1 ml/min at ambient temperature and the injection volume used was 20µl. The detection was carried out at 240 nm. Diluent was prepared by mixing 500 ml of methanol, 500 ml of Acetonitrile, filtered through membrane filteration (0.22µm) and degassed before use. Typical chromatogram of standard drugs is as shown in Fig. 3.

Preparation of mobile phase

The mobile phase was prepared by mixing 500 ml of methanol, 500 ml of Acetonitrile, filtered through membrane filtration (0.22µm) and degassed before use.

Preparation of stock solution

Accurately weighed quantity of each drug (Amlodipine Besylate 10 mg and Telmisartan 80mg) was transferred to 100 ml volumetric flask. Then small amount of methanol was added and ultra-sonicated for 5 min and diluted up to the mark with methanol.

Construction of calibration curve

From the stock solution 0.5, 1, 2, 4, 6, 8 ml were pipette out into 10 ml volumetric flasks and diluted to final volume using methanol as solvent. That gave 5, 10, 20, 40, 60, 80 µg/ml of Amilodipine Besylate and 40, 80, 160, 320, 480, 640 µg/ml of Telmisartan. From this solution 20 µl was injected into HPLC system and peak area were noted. The calibration curves were constructed by taking concentration of drug in X axis and peak area in Y axis.

Preparation and analysis of sample solution

20 tablets of AMLOPRES TL (containing 5mg of Amlodipine and 40mg of Telmisartan) were weighed and average weight was calculated and powdered. Weighed accurately powder equivalent to 5mg of Amlodipine and 40mg of Telmisartan and transferred to 10ml volumetric flask. Then small amount methanol was added into the volumetric flask, sonicated for about 15min, and the final volume was made with same to obtain solution having the concentration of 500µg/ml of Amlodipine and 4000 µg/ml of Telmisartan. The mixture was then filtered through Membrane filtration (0.45µm). The above solution was suitably diluted with mobile phase to obtain the solution having the final concentration of 20 µg/ml of Amlodipine and 160 µg/ml of Telmisartan. A typical chromatogram formulation (sample) drug was shown in Fig. 4. The assay results are shown in Table 2.

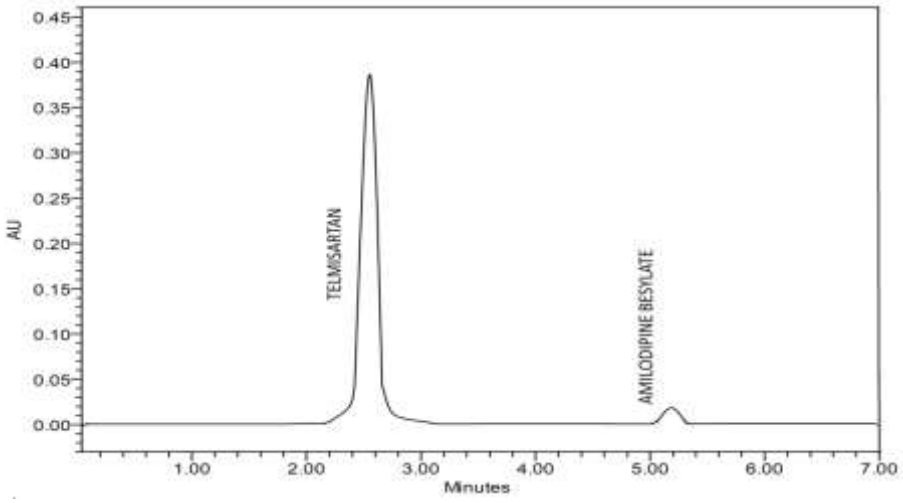

Figure 3: Typical chromatogram of standard drugs

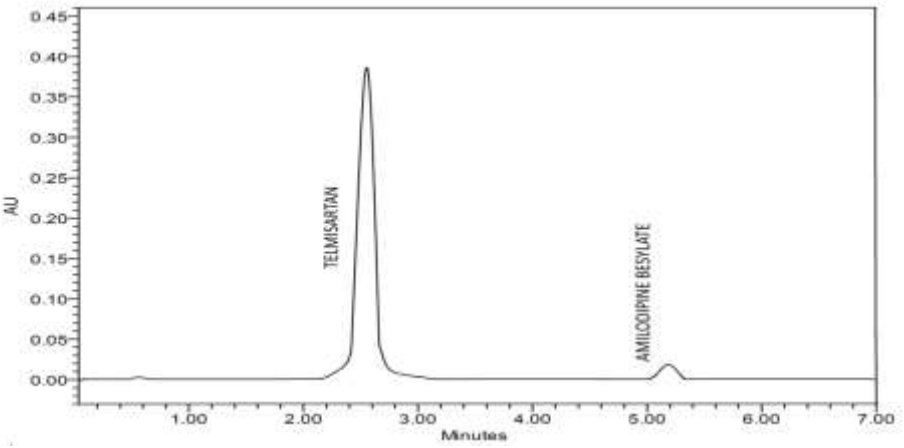

Figure 4: Typical chromatogram formulation

Table 1: Validation and system suitability parameters

Parameters	Amlodipine	Telmisartan
Linearity range (µg/ml)	5-80µg/ml	40-640µg/ml
Slope (m)	3356	42097
Intercept (c)	595.9	1917
Correlation coefficient (r^2)	0.999	0.999
Retention time (min)	2.5	5.2
% Recovery	99.52-100.90	99.68-100.22
Tailing factor	1.41	1.32
Theoretical plates	≥3920	≥2860
LOD (µg/ml)	0.0398	0.0039
LOQ (µg/ml)	0.1206	0.1182

Table 2. Assay and precision studies

Drug	Label claim mg/tab	Amount found (n=5)		Intraday % RSD	Inter day % RSD	
		mg/tab	%		Day 1	Day2
Amlodipine	5 mg	4.99	99.97±0.085	0.2151	0.1939	0.2015
Telmisartan	40 mg	39.99	99.9±0.0012	0.1897	0.1647	0.1825

RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phases of different compositions were tried. A satisfactory separation and good peak symmetry for Amlodipine Besylate and Telmisartan were obtained with a mobile phase consisting of Acetonitrile: Methanol (50: 50 v/v). Quantification was achieved with UV detection at 240nm based on peak area. Complete resolution of the peaks with clear baseline was obtained. System suitability parameters was calculated and compared with the standard limit as per ICH. The method was validated for its linearity range, accuracy, precision, sensitivity and specificity. The results for validation parameters are in Table 1.

Linearity

Calibration curves were constructed by plotting peak area Vs concentration of drug solutions and the regression equation were calculated. The calibration curves were plotted over the concentration range 5-80µg/ml for Amlodipine Besylate and 40-640µg/ml for Telmisartan. The diluted final concentration solutions of each drug were prepared carefully from their working standard solutions. Each 20µl aliquots of solution were injected into the HPLC system which is operated according to chromatographic condition as described above. The constructed calibration curves of Amlodipine Besylate and Telmisartan were shown in Fig. 5 and 6.

Accuracy

The accuracy of the methods was determined by calculating recovery values of Amlodipine Besylate and Telmisartan by the standard addition method. The accuracy of the method was determined at various level 50%, 100% and 150%. That was carried out by preparing solutions of different concentrations in which the amount of marketed formulation (AMLOPRESS TL) was varied (5mg of Amlodipine and 40mg of Telmisartan) and the amount of pure drug was kept constant. The solutions were prepared in triplicates and the accuracy was indicated by %. The results are in table 1.

Precision

The precision of the instruments was checked by repeatedly injecting solutions of Amlodipine Besylate and Telmisartan. The intra-day and inter-day precision of the proposed methods were determined by the corresponding responses 6 times on the same day and on 2 different days.

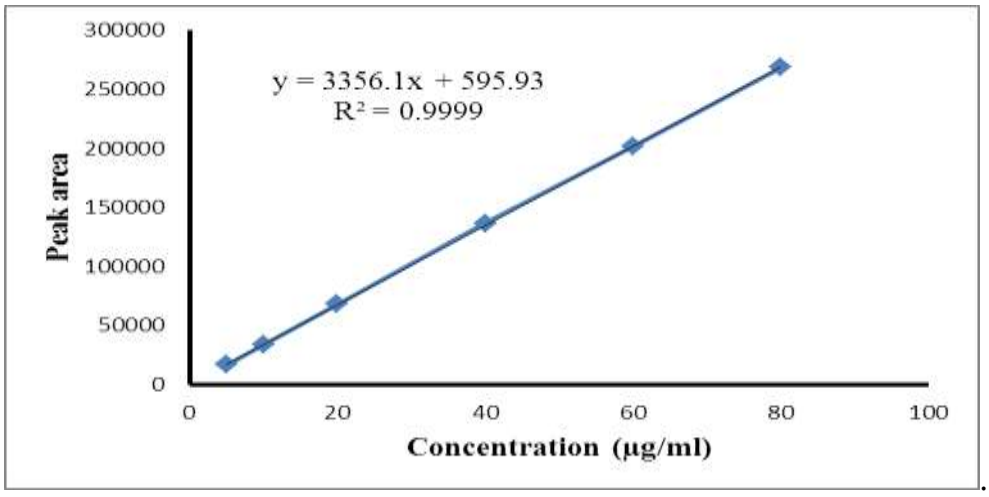

Figure 5: Calibration curve of Amlodipine Besylate

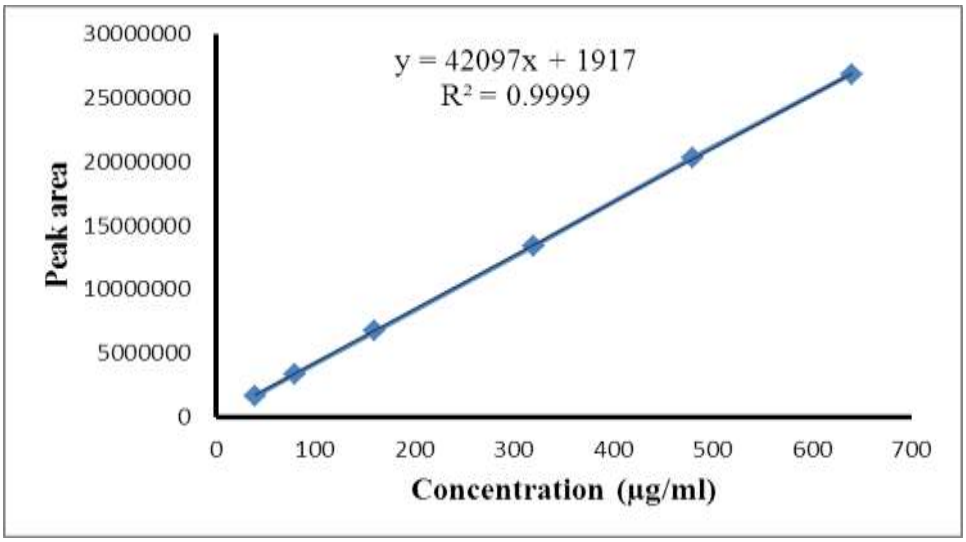

Figure 6: Calibration curve of Telmisartan

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were calculated using the following equations as per International Conference of Harmonization (ICH) guidelines.

$LOD = 3.3 \times \alpha/S$

$LOQ = 10 \times \alpha/S$

CONCLUSION

A new, reversed-phase HPLC method has been developed for simultaneous analysis of Amilodipine and Telmisartan in a tablet formulation. It was shown that, the method was linear, accurate, reproducible, repeatable, precise, selective and specific proving the reliability of the method. The run time is relatively short, i.e. 7 min, which enable rapid determination of any samples in routine and quality control analysis of tablet formulations. The same solvent was used throughout the experimental work and no interference from any excipient was observed. Hence, the proposed method was successfully applied to analyze the tablet formulation containing Amilodipine and Telmisartan.

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