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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND TELMISARTAN IN TABLET DOSAGE FORM

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ABSTRACT

A simple, sensitive and rapid reverse phase HPLC method was developed for the simultaneous estimation of Amlodipine besylate and Telmisartan. A Phenominex-luna C_{18} column (250x4.6 mm i.d 5µ) was used with a mobile phase containing a mixture of acetonitrile and phosphate buffer in the ratio of 56:44%v/v.pH was adjusted with orthophosphoric acid to 4.The flow rate was 1ml/min and the eluents were monitored at the detector wavelength of 236nm.The retention times of Amlodipine besylate and Telmisartan were found to be 4.32 and 5.32 minutes respectively.The validation of the proposed method was carried out for its specificity, accuracy, pecision, linearity, limit of detection and limit of quantification for both Amlodipine and Telmisartan.

Keywords: Amlodipine besylate, Telmisartan, Reverse phase HPLC.

INTRODUCTION

Amlodipine besylate is a calcium channel blocker, chemically it is 2-[(2-amino ethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine - dicarb oxylic acid 3-ethyl,5-methyl ester and Telmisartan is 2-(4-{(4-methyl-6-(1-methyl-1H-1,3- benzodiazole-2-yl) -2-propyl-1H-1,3benzodiazole-1-yl]methyl}phenyl) benzoic acid.Amlodipine used as an anti hypertensive and in the treatment of angina.Amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing total peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart muscle.Telmisatan is an angiotensin-II receptor blocker that shows high affinity for the angiotensin II receptor type 1(AT_1) with a binding affinity 3000 times greater for AT_1 than AT_2. Telmisartan lowers blood pressure through blockade of renin-angiotensin-aldosterone system (RAAS) and is widely used in the hypertension [1,2].

The literature reveals that there are some of the methods have been reported for simultaneous estimation of amlodipine besylate and telmisartan by UV spectrophotometry [3-5], HPTLC and RP-HPLC [6,7]. Most of the literatures are the simultaneous estimation of amlodipine and atorvastatin by RP-HPLC for telmisartan development and validation of RP-HPLC method for the simultaneous estimation of hydrochlorthiazide and telmisartan. An attempt was made to develop and report a simple, sensitive, validated and economic method for the simultaneous determination of amlodipine besylate and telmisartan in tablet dosage form by HPLC.

MATERIALS AND METHODS

Reagents

Acetontrile and water (HPLC grade), disodium hydrogen phosphate (AR grade),methanol(AR grade), orthophosphoric acid and distilled water. Commercial samples of tablets containing the drug were purchased from the local pharmacy.

Equipments and apparatus

Different kinds of equipment like Shimadzu

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Electronic Balance, Elico p^H Meter, Ultra Sonicator, Millipore-solvent Filtration unit, Shimadzu UV-visible Spectrophotometer, Waters HPLC system containing injector phenomenex-luna,C_{18}(250x4.6mm i.d.,5µ) as analytical column were used throughout the experiment.

Chromatographic conditions

Analysis was carried out at 236nm using a Phenominex Luna C_{18} (250 x 4.6mm, 5µm) at room temperature. The mobile phase consists of acetonitrile and phosphate buffer in the ratio of 56:44%v/v (p^H 4) and the flow rate was set at 1ml/min.

Preparation of buffer

Weigh and transfer about 3.8954g of disodium hydrogen phosphate and 3.4023g of potassium dihydrogen phosphate into a beaker containing 1000ml distilled water and dissolve completely. The pH of the solution was adjusted to 4±0.01.with orthophosphoric acid and then filtered through 0.45µm membrane filter.

Preparation of mobile phase

Mobile phase was prepared by mixing 560ml of acetonitrile and 440ml of buffer (56:44).Then it was sonicated using ultra sonicator to remove the impurities and dissolved gases, as they lead to unwanted peaks in the chromatogram.

Diluent preparation

Use the mobile phase as diluent.

Preparation of standard stock solution

Accurately weighed quantity of 5mg of Amlodipine besylate and 40mg of Telmisartan were transferred into a 100ml volumetric flask, add 25ml of mobile phase and sonicate till dissolved .Then diluted to required volume with mobile phase.

Preparation of standard solution

From the standard stock solution 5ml was pipetted out into 100ml volumetric flask and made up to the volume with mobile phase.

Preparation of sample solution

Twenty tablets were weighed and ground to a fine powder. An amount of powder equivalent to 40mg of Telmisartan and 5mg of Amlodipine besylate were weighed accurately and transferred into a 100ml volumetric flask. Add 25ml of mobile phase to it and sonicated for 30min.Then it was diluted to 100ml with mobile phase and filtered through 0.45µm membrane filter. From this solution 5ml of filtrate was taken into 100ml volumetric flask and made up to the volume with mobile phase.

METHOD VALIDATION

The proposed method was validated as per ICH guidelines. The drug solutions were prepared as per procedure given in the experiment.

Fig.1. Structure of Amlodipine besylate

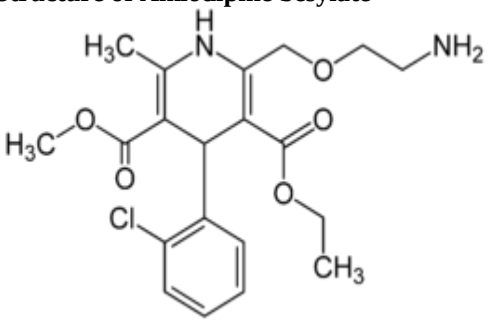


Fig.2. Structure of Telmisartan

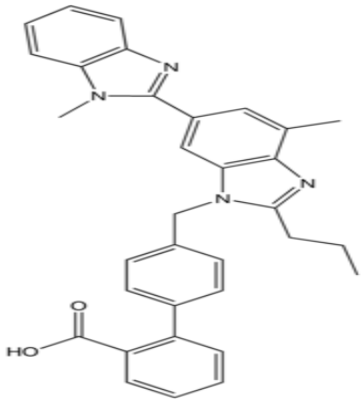
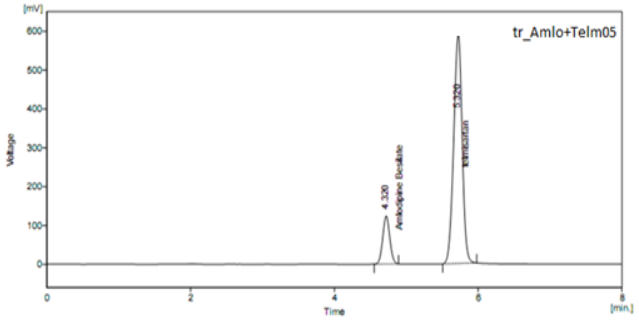


Fig. 3. Optimized chromatogram of Amlodipine besylate and Telmisartan by RP-HPLC



RESULTS AND DISCUSSIONS

A reverse phase HPLC method was proposed as a suitable method for the simultaneous determination of Amlodipine and Telmisartan in combined dosage form. The chromatographic conditions were optimized by changing the mobile phase composition, pH and buffers used in the mobile phase. To optimize the mobile phase different trials were experimented on different ratios. The results from development activity are that suitable, easy, less time consuming validated method was developed for the simultaneous determination of Amlodipine and

Telmisartan by RP-HPLC.

Fig 4. Linearity plot for Amlodipine besylate

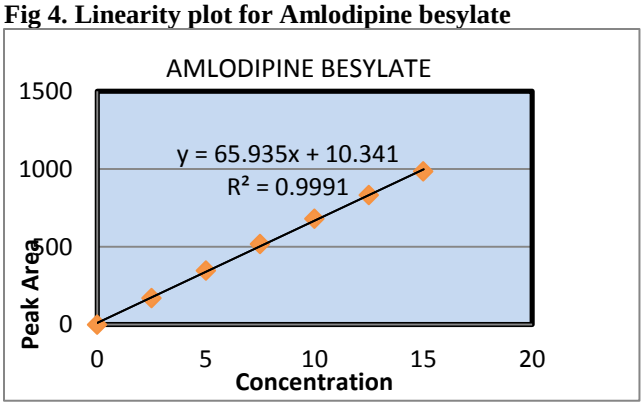
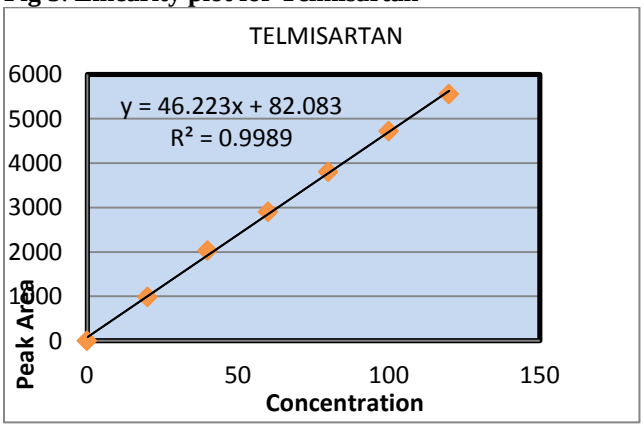


Fig 5. Linearity plot for Telmisartan



Linearity and Range

The linearity of calibration curves in pure solution was checked over the concentration ranges of 2.5-1.5µg/ml for the Amlodipine and 20-120µg/ml for Telmisartan respectively. The linearity was evaluated by linear regression analysis using least squares method. The slope, intercept and correlation coefficient values for Amlodipine were found to be 65.93, 10.34 and 0.999 respectively. The slope, intercept and correlation coefficient values for Telmisartan were found to be 46.22, 82.08 and 0.998 respectively.

Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the assay was determined by repeatability (inter day) and intermediate precision (inter day).The precision of test method was done by performing assay on five replicate determination of sample preparation at test concentration level and calculated relative standard deviation of assay results. System precision and method precision were determined.

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ were determined from the calculated standard deviation of each calibration standard. The LOD was found to be 0.04791µg/ml and 0.04496µg/ml for Amlodipine and Telmisartan respectively and the LOQ was found to be 0.1452µg and 1.3625µg for Amlodipine and Telmisartan respectively.

Table 1. Linearity data for Amlodipine and Telmisartan

Concentration of Amlodipine Besylate (µg/ml)	Peak Area of Amlodipine Besylate(Mv)	Concentration of Telmisartan µg/ml)	Peak Area of Telmisartan (Mv)
2.5	169.754	20	987.511
5.0	347.523	40	2031.509
7.5	518.159	60	2899.555
10.0	680.211	80	3800.012
12.5	832.696	100	4717.711
15.0	985.623	120	5552.031

Table 2. Recovery studies for Amlodipine and Telmisartan

S No	Inj.Sample	Spike level	Amount Present	Amount Recovered	% Recovered
1	**Amlodipine**	80 %	10mcg	9.9765	99.765%
2		100 %	12.5mcg	12.471	99.77%
3		120 %	15mcg	14.9558	99.70%
4.	**Telmisartan**	80 %	80mcg	79.968	99.60%
5		100 %	100 mcg	99.951	99.99%
6		120 %	120mcg	119.872	99.89%

Table 3. System precision of Amlodipine and Telmisartan

S.NO	Area of Amlodipine (mV)	Area of Telmisartan (mV)
1	804.202	4664.222
2	806.602	4665.943
3	816.968	4562.585
4	815.694	4663.066
5	817.332	4664.558
Mean	812.1596	4642.075
S.D	6.256531	44.65221
R.S.D	0.770357	0.961902

Table 4. Method precision of Amlodipine and Telmisartan

Sample No	Area of Amlodipine (mV)	Area of Telmisartan (mV)
1	833.187	4565.943
2	826.814	4664.222
3	828.435	4572.585
4	811.505	4663.066
5	822.505	4654.558
Mean	824.489	4642.075
S.D	8.20293	44.65221
R.S.D	0.9949105	0.961902

Table 5. Robustness for Amlodipine and Telmisartan

Effect	Retention time of Amlodipine	Retention time of Telmisartan
P^H (3.8)	4.667	5.633
P^H (4.2)	4.660	5.620
Temp(23ºc)	4.567	5.640
Temp(30ºc)	4.260	5.227

Accuracy

To study reliability, suitability and accuracy of the method recovery studies were carried out by adding a known quantity of the standard to the pre analysed sample. The recovery was carried out at 80%, 100% and 120% level. From the respective chromatogram the contents were determined.

Robustness

For demonstrating the robustness of the developed method experimental conditions were altered and evaluated. The method must be robust to withstand slight changes in chromatographic conditions and allow routine analysis of the sample. Effect of column temperature and effect of buffer p^H were carried out and standard was injected.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of Amlodipine besylate and Telmisartan. Different chromatographic conditions were used to develop the method. Elution was carried out with a mobile phase consists of acetonitrile: phosphate buffer in the ratio of 56:44%V/V at p^H 4, and the flow rate was 1ml/min. The retention times for Amlodipine and Telmisartan were found to be 4.32 and 5.32 respectively. The solvents used are economic and easily available and hence the newly developed method can be used for routine analysis for the simultaneous estimation of Amlodipine besylate and Telmisartan in tablet dosage form.

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