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RESEARCH ARTICLE

VALIDATED RP – HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TELMISARTAN AND AMLODIPINE

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Abstract:
A simple, sensitive and rapid reverse phase HPLC method was developed for the simultaneous estimation of Telmisartan and Amlodipine. AnHypersil BDS 100mm x 4.6 mm, 5µ column was used. The mobile phase consisting of Phosphate buffer (pH 3.5): Acetonitrile taken in the ratio 57: 43 that was set at a flow rate of 1.0 mL/min, the column temperature was maintained at 30ºC and the measurements were made at 237 nm. The retention times of Telmisartan and Amlodipine were found to be 2.560 and 3.148 min, respectively.The validation of the proposed method was carried out for its specificity, accuracy, precision, linearity, limit of detection and limit of quantification for both Amlodipine and Telmisartan.

Keywords: Amlodipine besylate, Telmisartan, Reverse phase- HPLC.

Introduction

The combinations of two or more drugs in the pharmaceutical dosage forms are very much useful in multiple therapies. Market survey revealed that, day-by-day new drugs and their combination with another drugs are being introduced in market as they have more patient compliance than a single drug[1].Telmisartan, 4-[{2-n-propyl-4-methyl-6-(1-methyl benzimidazol-2-yl)-benzimidazol-1-yl} methyl]–bi phenyl-2-carboxylic acid is a new highly selective, nonpeptide angiotensin II type 1 (AT1)-receptor antagonist. Telmisartan lowers blood pressure through blockade of the rennin-angiotensin-aldosterone system (RAAS) and is widely used in the treatment of hypertension[2,3].

Amlodipine, 2[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine carboxylic acid, 3-ethyl,5-methylester besylate is a dihydropyridine derivative with calciumantagonist activity [4,5], used mainly as an antihypertensive andantianginal agent.

The literature reveals that there are some of the methods have been reported for simultaneous estimation of amlodipine besylate and telmisartan by UV spectrophotometry [6-8], HPTLC and RP-HPLC [9,10]. 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 and telmisartan by HPLC.

MATERIALS AND METHODS

Instrumentation:

The HPLC system consist of a Waters Alliance equipped with a Waters 2695 separations module, loop of injection capacity of 10µL and a Waters 2996 PDA detector. Data acquisition was performed by the Empower- 2 software. Analysis was carried out at 237 nm with a reversed phase Hypersil BDS 100mm x 4.6 mm, 5µ.

Chemicals and Reagents:
Telmisartan and Amlodipine standard substances were gifted by Spectrum pharma lab, Hyderabad. HPLC Grade Water (Merck), HPLC grade Acetonitrile (Merck), O-phosphoric acid of AR Grade (Rankem) and were used during the study.

Chromatographic Conditions:
Chromatographic separations were achieved using Column Hypersil BDS 100mm x 4.6 mm, 5µ. The mobile phase consisting of Phosphate buffer (pH 3.5): Acetonitrile taken in the ratio 57 : 43 that was set at a flow rate of 1.0 mL/min. the column temperature was maintained at 30ºC. The mobile phase was degassed by ultra sonication before pumping into HPLC system. The flow rate was maintained at 1mL/min and the measurements were made at 237 nm.

Preparation of Buffer (0.01N Phosphate buffer):
Accurately weighed 1.36gm of Potassium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.5 with dil. Orthophosphoric acid solution.

Preparation of Mobile phase:
Mobile phase was prepared by mixing 570ml of buffer and 430ml of acetonitrile (57:43). Then it was sonicated to remove the impurities nad dissolved gases, as they lead to unwanted peaks in the chromatogram.

Preparation of Standard Stock Solution:
Accurately Weighed quantity of 40mg of Telmisartan and 5mg of Amlodipine working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents.

Preparation of working standard solution:
From the above stock solution, 1 ml was pipeted out in to a 10ml volumetric flask and then make up to the final volume with diluent.

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

RESULTS AND DISCUSSION
Method Development:
An aliquot containing 10µl of Telmisartan and Amlodipine drug solutions were injected into HPLC system. The mobile phase was chosen after several trials with Acetonitrile, methanol, potassium phosphate buffer and other buffer solutions in various proportions. The mobile phase of phosphate buffer: acetonitrile at different ratios were used, but yielding not proper peak shapes with fronting and tailing in the peak. Followed by, Potassium phosphate buffer (pH 3.5): Acetonitrile (57:43v/v) was used as mobile phase. The chromatogram was improved and sharp peaks, in good shapes and symmetrical were obtained. It was concluded that the best HPLC parameters for the simultaneous detection of Telmisartan and Amlodipine was at 237 nm by using Potassium phosphate buffer (pH 3.5): Acetonitrile (57:43v/v). The flow rate was at 1.0 ml/min and the injection volume was 10µl. The retention time of Telmisartan and Amlodipine were 2.560 and 3.148 min, respectively. The optimized method was validated as per ICH guidelines.

Method Validation

Linearity:
The linearity of calibration cure in pure solution was checked over the concentration range of of 100ppm – 600ppm for telmisartan and 12.5ppm – 75ppm for amlodipine. The linearity was evaluated by linear regression analysis using least squares method. The slope, intercept and correlation coefficient values for Telmisartan were found to be 20178, 3803 and 0.999 respectively. The slope, intercept and correlation coefficient values for Amlodipine were found to be 19566, 749 and 0.999 respectively.

Limit of Detection (LOD) and Limit of Quantification (LOQ):
The LOD and LOQ were determined from the calculated standard deveiation od each calibration standard. The LOD was found to be 0.62µg/ml and 0.13µg/ml for Telmisartan and Amlodipine respectively. The LOQ was found to be 1.88µg/ml and 0.38µg/ml for Telmisartan and Amlodipine 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.

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 50%, 100% and 150% level. From the respective chromatogram the contents were determined.

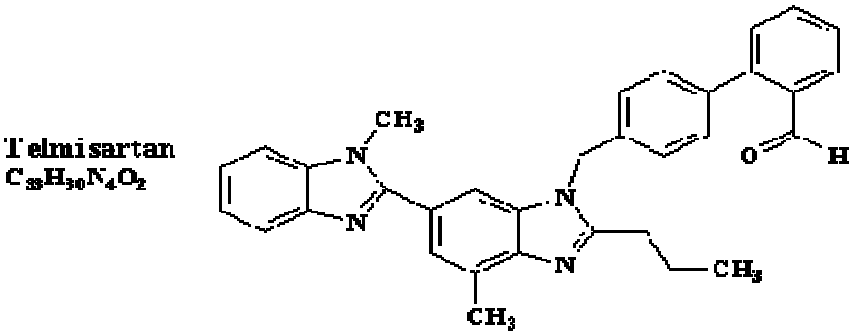

Fig -1: Structure of Telmisartan

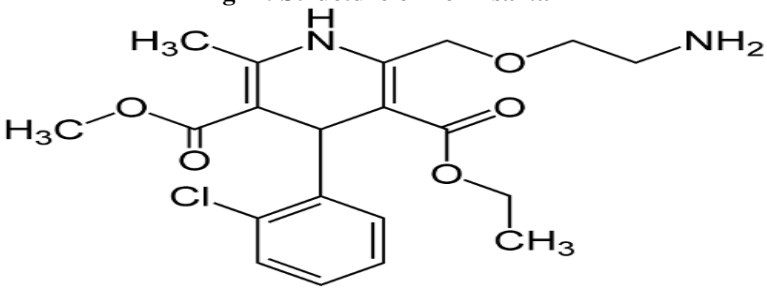

Fig-2: Structure of Amlodipine

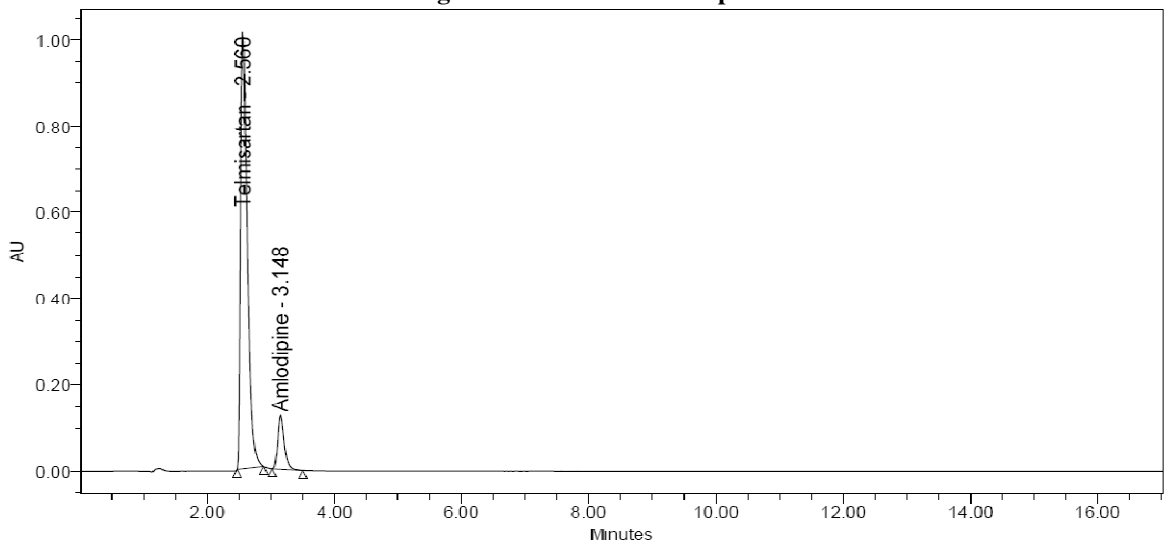

Fig- 3: Developed Chromatogram for Telmisartan and Amlodipine by RP – HPLC

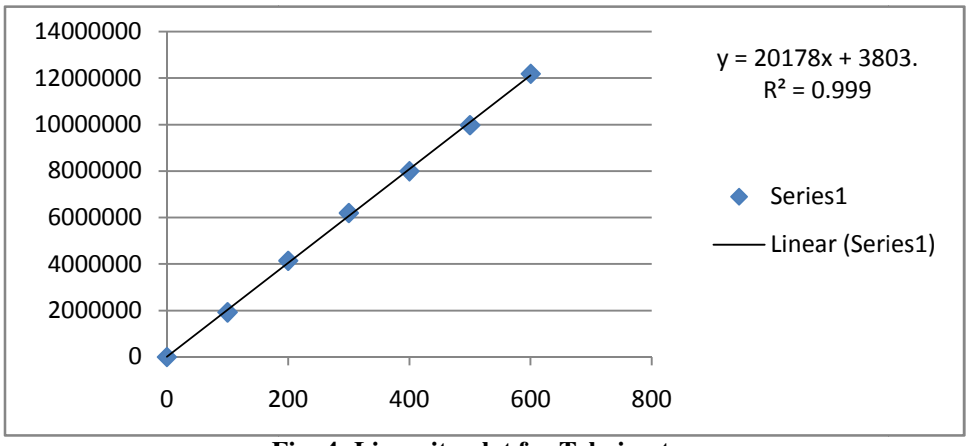

Fig- 4: Linearity plot for Telmisartan

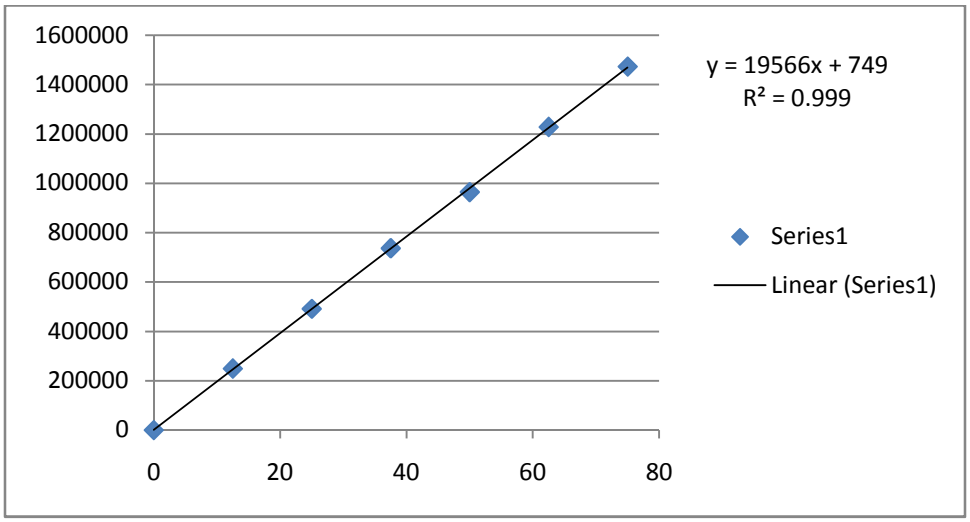

Fig- 5: Linearity plot for Amlodipine

Table- 1: Linearity Data for Telmisartan and Amlodipine

Concentration of telmisartan (ppm)	Peak Area of telmisartan	Concentration of Amlodipine (ppm)	Peak Area of Amlodipine
100	1921024	12.5	249104
200	4138621	25	491149
300	6195206	37.5	736530
400	7992503	50	964081
500	9972516	62.5	1228061
600	12180477	75	1472438

Table- 2: System Precision for Telmisartan and Amlodipine

S. No	Area of Telmisartan	Area of Amlodipine
1	7274556	863356
2	7312099	860242
3	7332339	867831
4	7286078	874449
5	7321327	877949
Mean	7305280	868765
S. D	24232.5	7406.8
% RSD	0.3	0.9

Table- 3: Method Precision for Telmisartan and Amlodipine

S. No	Area of Telmisartan	Area of Amlodipine
1	7468183	890024
2	7459997	890063
3	7479697	894162
4	7451313	892963
5	7443421	899714
Mean	7460522	893385.2
S. D	14176.54	3973.98
% RSD	0.19	0.44

Table-4:Results of % Recovery studies of telmisartan

Inj. Sample	Spike Level	% recovered	%Mean recovery	Standard Deviation	%RSD
Telmisartan	50 % 50% 50%	100.51% 99.45% 99.39%	99.78%	0.630	0.631
	100 % 100% 100%	99.25% 99.42% 99.92%	99.53%	0.348	0.350
	150 % 150% 150%	100.51% 100.83% 100.39%	100.58%	0.229	0.228

Table- 5:Results of % Recovery studies of amlodipine

Inj. Sample	Spike Level	% recovered	Mean recovery	Standard Deviation	%RSD
Amlodipine	50 % 50% 50%	99.60% 100.34% 100.27%	100.07%	0.4079	0.4076
	100 % 100% 100%	99.99% 100.82% 100.25%	100.35%	0.4261	0.424
	150 % 150% 150%	99.80% 100.07% 99.71%	99.86%	0.1863	0.1865

Table- 6: List of system suitability parameters of telmisartan and amlodipine

Sl. No	Parameters	Telmisartan	Amlodipine
1	Tailing Factor	1.74	1.43
2	Retention Time	2.510	3.072
3	Theoretical plates	2733	4488
4	Average Area	7452941	890002.6
5	%RSD	0.46	0.580847

System suitability testing

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated and the parameters like tailing factor, retention time, theoretical plates per unit, resolution factor are 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 pH 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 phosphate buffer: acetonitrile in the ratio of 57:43%V/V at pH 4, and the flow rate was 1ml/min. The retention times for Telmisartan Amlodipine and were found to be 2.560 and 3.148 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 Telmisartan and Amlodipine.

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