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Research Article

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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TELMISARTAN AND ATORVASTATIN CALCIUM IN TABLET DOSAGE FORMS

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ABSTRACT

The present research work deals with the development of RP-HPLC method for the determination of Telmisartan and Atorvastatin calcium in bulk and in formulation using UV detector. Selected mobile phase was a combination of Acetonitrile: Buffer (0.01M Potassium dihydrogen phosphate) 65:35 PH 4.00 (adjusted with Orthophosphoric acid) and the wavelength selected was 250nm. The flow rate was kept at 2.0 ml/min, and the injection volume was 10µl. The sepration was performed at ambient temperature. Retention time of Telmisartan and Atorvastatin calcium was found to be 3.72 and 6.14 minutes respectively. Linearity of the method was found to be 319-480 µg/ml for Telmisartan and 86-130 µg/ml for Atorvastatin calcium. The correlation co-efficient of Telmisartan was found to be 0.9998 and the correlation co-efficient of Atorvastatin calcium was found to be 0.9999. Accuracy of the method was determined through recovery studies by adding known quantities of standard drug to the pre analyzed test solution and was found to be 98.92-100.02 for Telmisartan and 99.93-100.96 for Atorvastatin calcium respectively The system suitability parameters such as theoretical plates and tailing factor were found to be 6347, 1.652 and 9720, 1.394 respectively for Telmisartan and Atorvastatin calcium. This method was validated according to ICH guidelines.

Keywords: Telmisartan and Atorvastatin calcium and RP-HPLC.

INTRODUCTION

Telmisartan is a non peptide molecule. It is chemically described as 4' – [(1, 4'-dimethyl-2' propyl [2, 6' bi-1H benzimidazole] – 1'-yl) methyl] – [1, 1' biphenyl] 2-carboxylic acid. Telmisartan is a non peptide angiotensin II receptor antagonist which selectively and insurmountably inhibits angiotensin II AT_1 receptor subtype without affecting other systems involved in cardiovascular regulation.Telmisartan blocks the vasoconstrictor and aldosterone secretion effect of angiotensin II by selectively blocking the binding of angiotensin II to the AT_1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland. Its action is therefore independent of the pathways for angiotensin II synthesis. AT_2 receptor is found in many tissues. But AT_2 is not known to be associated with cardio vascular homeostasis. Telmisartan has greater affinity (>3,000 fold) for AT_1 receptor than for the AT_2 receptor.Atorvastatin is a synthetic lipid – lowering agent. It is chemically designed as [R – (R*, R*)] – 2- (4- flurophenyl) β-dihydroxy – 5 - (1-methyl ethyl) – 3-phenyl-4-[(phenylamino) carbonyl] -1H-pyrole – 1-heptanoic acid, calcium salt (2:1) trihydrateAtorvastatin is a synthetic lipid-lowering agent.

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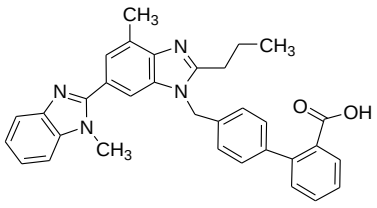
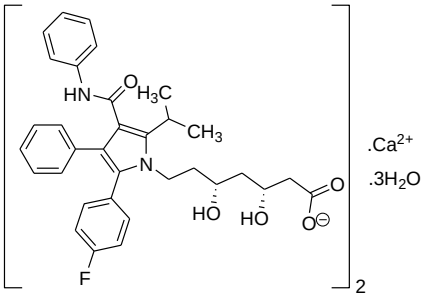
It is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase enzyme. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate limiting step in the synthesis of cholesterol.The primary site of action of HMG-CoA reductase inhibitors is the liver. Inhibition of cholesterol synthesis in the liver leads to up regulation of LDL-receptors and an increase in LDL-catabolism. There is also some reduction of LDL-production as a result of inhibition of hepatic synthesis of very low density lipoprotein (VLDL), the precursor of LDL-cholesterol. For the present study the combination of Telmisartan and Atorvastatin calcium was selected. The extensive literature survey carried out revealed that there is no method reported for the simultaneous estimation of these drugs, some methods for estimation of individual drugs by HPLC[1-11] ,HPTLC[12-14], Spectrophotometry[15-20] and LC-MS[21-22] are available. Hence present study aim to developing a specific, precise, acoustic, linear, simple, rapid, validated and cost effective RP-HPLC method for the simultaneous estimation of these drugs in combined dosage forms.



Telmisartan



Atorvastatin calcium

MATERIALS AND METHODS

Material

Working standards of Telmisartan and Atorvastatin calcium were received as gift samples from Dr. Ceeal Analytical Lab, Chennai. Tablets were purchased at Service Medicals, Vadakara. Distilled Water Acetonitrile HPLC grade.Potassium di hydrogen phosphateOrthophosphoric acid. Shimadzu LC-20 AT HPLC Detector UV visible (SPD 20A)pH meter Advanced instruments Melter electronic balanceSonicator sandelin – sonorex super Rx-106.

Chromatographic System and Conditions

Stationary phase: Phenomenex C_{18} column (250x4.6mm i.d, 5µm)
Mobile phase: Acetonitrile: Buffer (0.01M Potassium dihydrogen phosphate)
PH : 4.00 (adjusted with Orthophosphoric acid)
Solvent ratio: 65:35
Detection wavelength: 250 nm
Flow rate: 2.0ml/min
Temperature: Room temperature

Preparation of mobile phase

Acetonitrile and buffer were mixed in the ratio of 65:35 and filtered through membrane filter and degassed in a sonicator for 10 minutes.

Preparation of buffer (0.01M)

1.3609 gm of Potassium di-hydrogen phosphate in sufficient water to produce 1000ml, pH adjusted to 4.0 with orthophosphoric acid.

Preparation of standard solution

Weight accurately about 399.6mg of Telmisartan and 108.3mg of Atorvastatin calcium and transferred in to a clean 100 ml volumetric flask dissolved in few ml of methanol and make up to the volume with methanol. Sonicate for 10 minutes and filtered through membrane filter and marked as standard stock solution Pipette out 5ml from the standard stock solution into a clean 50ml standard flask and make up the volume 50 ml with mobile phase and marked as standard stock solution A.

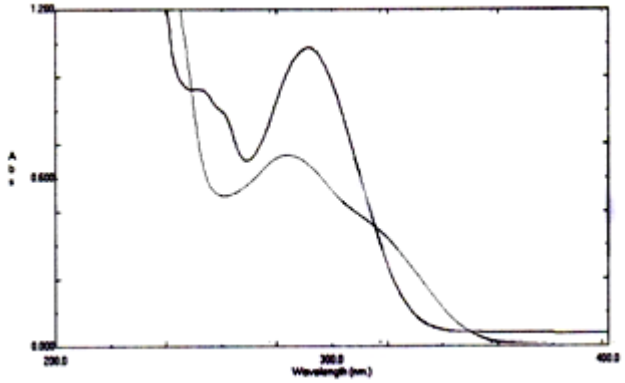
Preparation of sample solution

Weigh and powder 20 tablets, weight accurately a quantity of powder equivalent to 100 mg of Atorvastatin calcium and transfered it into a clean 100 ml standard flask. Add few ml of methanol and dissolved, make up the volume with methanol. The solution is sonicated for 10 minutes and filtered through membrane filter, and marked as sample stock solution. Pipette out 5 ml from the sample stock solution in to a clean 50 ml standard flask and make up the volume 50 ml with mobile phase. So as to give a concentration of 40 mg of Telmisartan and 10 mg Atorvastatin calcium.

Method development and Optimisation

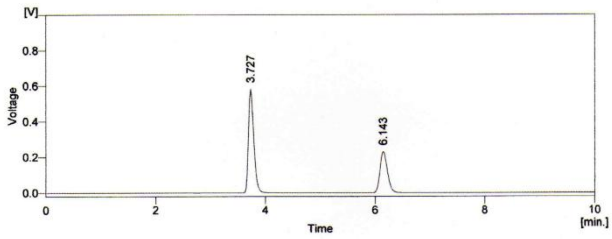
The wavelength for the analysis of was selected from the UV spectrum of Telmisartan and Atorvastatin calcium by scanning in the range of 200-400nm. From this, the wavelength of 250nm was selected for the final method as these drugs has shown good absorbances. 3D view of the combined spectra using PDA detector was observed, the maximum absorbance with good peak intensity, good peak shape and height was observed at 250nm. (Figure **1**)

Figure 1.Overlay absorption spectra Telmisartan and Atorvastatin calcium



For HPLC analysis, initially various mobile phases and stationary phases were tried in attempts to obtain the best separation and resolution between Telmisartan and Atorvastatin calcium. The mobile phase consisting a combination of acetonitrile and 0.01M potassium di hydrogen phosphate buffer of pH 4.0 in the ratio of 65:35 v/v.was found to be an appropriate mobile phase allowing adequat separation of two drugs using a C18 phenomenex Gemini 5µ, 250cm x 4.6mm id with flow rate of 2.0ml/min using PDA detection at 250nm. A typical chromatogram of separation of two compounds is shown in (Figure **2**)

Figure 2. HPLC Chromatogram of Telmisartan and Atorvastatin calcium



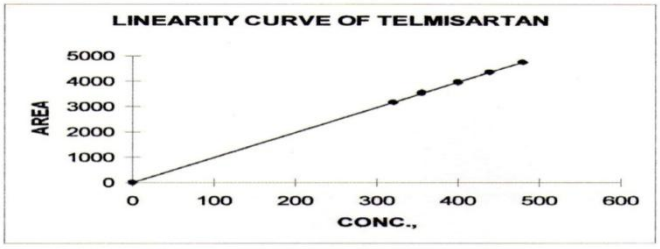
Methode validation

The developed method was validated for simultaneous assay determination of Telmisartan and Atorvastatin calcium using following parameters.

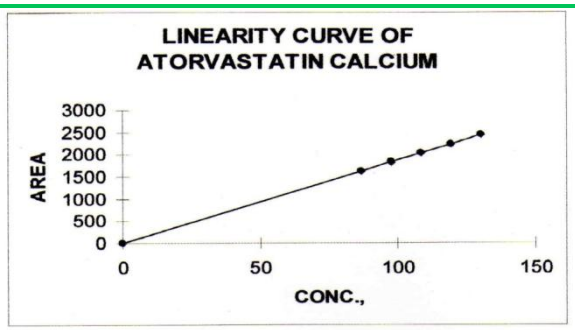
Linearity

Linearity was demonstrated by analysing six different concentrations of active compound. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area vs concentrations of Telmisartan and Atorvastatin calcium which were found to be linear in the range of 319-480 µg/ml and 86-130 µg/ml respectively. Coefficient of correlation was 0.9998 and 0.9999 (Figure **3**).

Figure 3. Linearty curve for Telmisartan and Atorvastatin calcium



LINEARITY CO-EFFICIENT 0.9998



LINEARITY CO-EFFICIENT 0.9999

Precision

To demonstrate agreement among results, a series of measurements are done with Telmisartan and Atorvastatin calcium six replicate injections of the specific standard at various time intervals on the same day were injected into the chromatograph and the value of %RSD was found to be 0.4163 and 0.4179 for Telmisartan and Atorvastatin calcium (Table **1**)

Table.1. Assay of Telmisartan and Atorvastatin calcium

Drug	Method Precision		Assay
	Mean %	RSD (%)	Amount found
Telmisartan	99.38	0.4163	39.75 mg
Atorvastatin Calcium	100.66	0.4179	10.06 mg

RESULTS AND DISCUSSION

Optimization of the mobile phase was performed based on resolution, asymmetric factor and peak area obtained for both Telmisartan and Atorvastatin calcium. The mobile phase combination of Acetonitrile: Buffer (0.01M Potassium dihydrogen phosphate) 65:35 PH 4.00 (adjusted with Orthophosphoric acid) found to be satisfactory and gave two symmetric and well resolved peaks for Telmisartan and Atorvastatin calcium. The retention time for Telmisartan and Atorvastatin calcium were 3.72 and 6.14, respectively (**figure 2**). The calibration curve for Telmisartan was obtained by plotting the peak area of Telmisartan versus the concentrations of Telmisartan over the range of 319-480 µg/ml , and it was found to be linear with r2 = 0.9998. Similarly, the calibration curve for Atorvastatin calcium was obtained over the range of 86-130 µg/ml and was found to be linear with r2 = 0.9999. The recoveries of Telmisartan and Atorvastatin calcium were found to be in the range of 99.81%-100.34% and 97.48%-98.39% within precision RSD of 0.4584 and 0.4563 for Telmisartan and Atorvastatin calcium. The system suitability parameters such as theoretical plates and tailing factor were found to be 6347, 1.652 and 9720, 1.394 respectively for Telmisartan and Atorvastatin calcium. The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The detection limit (LOD) was found to be 5.92µg/ml for Telmisartan and 0.82µg/ml for Atorvastatin calcium respectively.The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The quantitation limit (LOQ) was found to be 17.94µg/ml for Telmisartan and 2.50µg/ml for Atorvastatin calcium respectively. Proposed study describes a new RP-HPLC method for estimation of Telmisartan and Atorvastatin calcium combination in mixture using simple mobile phase. The method gives good resolution between both the compounds with a short analysis time. The method was validated and found to be simple, sensitive, accurate and precise. Percentage recovery shows that the method is free from interference of the excipients used in the formulation (Table **2**). Therefore, the proposed method can be used for routine analysis of Telmisartan and Atorvastatin calcium their combined dosage form.

Table 2.Validation and system suitability parameters

Parameters	Telmisartan	Atorvastatin Calcium
Linearity range µg/ml	319-480 µg/ml	86-130 µg/ml
Correlation Coefficient (r^2) ± S.D	0.9998	0.9999
Retention time (min) ± S.D	3.72	6.14
Resolution	11.096	11.096
Tailing factor	1.652	1.394
Theoretical Plate	6347	9720
Limit of detection (µg/ml)	5.92	0.82
Limit of Quantification (µg/ml)	17.94	2.5
Precision (RSD %) intraday (n=6)	0.4163	0.4179

CONCLUSION

In the current study a new RP-HPLC method for estimation of Telmisartan and Atorvastatin Calcium combination in mixture using simple mobile phase was developed, optimized and validated. The developed method is simple, sensitive, accurate and precise. The developed method can be used for routine analysis of Telmisartan and Atorvastatin Calcium in a combined dosage form.

REFERENCES

1. Shah D A, Bhatt K K, Mehta R S, Shankar M B, Baldania S L, Gandhi T R; Development and validation of a RP-HPLC method for determination of atorvastatin calcium and aspirin in a capsule dosage form, *Indian journal of pharmaceutical scinces*, 2007 vol 69 (4) Page : 546-549.
2. Shah D A, Bhatt K K, Mehta R S, Baldania S L, Gandhi T R; Stability indicating RP-HPLC estimation of atorvastatin calcium and amlodipine besylate in pharmaceutical formulations, *Indian journal of pharmaceutical scinces*, 2008 Vol 70 (6) Page : 754-760.
3. Palled M S, Rajesh P MN, Chatter M, Bhat A R; RP-HPLC determination of Telmisartan in tablet dosage forms, *Indian journal of pharmaceutical scinces*, 2005 Vol. 67 (1) Page : 108-110.
4. Raja K Rajeswari, Sankar G G, Rao A L, Seshagirirao JVLN; RP-HPLC method for the simultaneous

determination of Atorvastatin and Amlodipine in tablet dosage form, *Indian journal of pharmaceutical scinces,* 2006 Vol. 68 (2) Page : 275-277.

5. Wankhede S B, Tajne M R, Gupta K R, Wadodkar S G; RP-HPLC method for simultaneous estimation of telmisartan and hydrochlorothiazide in tablet dosage form, *Indian journal of pharmaceutical scinces*, 2007 Vol. 69 (2) Page : 298-300.
6. Shah D A, Bhatt K K, Mehta R S, Shankar M B, Baldania S L; RP-HPLC method for the determination of atorvastatin calcium and nicotinic acid in combined tablet dosage form, *Indian Journal of Pharmaceutical Sciences*, 2007 Vol. 69 (5) Page : 700-703.
7. Jain N, Raghuwanshi R, Jain D; Development and validation of RP-HPLC method for simultaneous estimation of atorvastatin calcium and fenofibrate in tablet dosage forms, *Indian journal of pharmaceutical scinces*, 2008 Vol.70 (2) Page : 263-265.
8. Shah D A, Bhatt K K, Shankar M B, Mehta R S, Gandhi T R, Baldania S L; RP-HPLC determination of atorvastatin calcium and amlodipine besylate combination in tablets, *Indian journal of pharmaceutical scinces* , 2006 Vol. 68 (6) Page : 796-799.
9. Patil K R, Rane V P, Sangshetti J N, Shinde D B; A Stability-Indicating LC Method for the Simultaneous Determination of Telmisartan and Ramipril in Dosage Form, *Chromatographia* 2008, 67, April (No. 7/8) Page no: 575–582.
10. Patil K R, Rane V P, Sangshetti J N and Shinde D B; A Stability-Indicating LC Method for the Simultaneous Determination of Telmisartan and Ramipril in Dosage Form, *Chromatographia* Volume 67, Numbers 7-8 April, 2008 ISSN 0009-5893 Pages 575-582.
11. Lucie N, Dalibor S, Petr S; HPLC methods for the determination of simvastatin and atorvastatin, *Trends in analytical chemistry* ISSN 0165-9936 2008, vol. 27, (4) pp. 352-367.
12. Chaudhari B G, Patel N M, Shah P B, Modi K P; Development and validation of a HPTLC method for the simultaneous estimation of atorvastatin calcium and ezetimibe, *Indian journal of pharmaceutical sciences,* 2006 Vol. 68 (6) Page : 793-796.
13. Shah N J, Suhagia B N, Shah R R, Shah P B; Development and validation of a HPTLC method for the simultaneous estimation of telmisartan and hydrochlorothiazide in tablet dosage form, *Indian journal of pharmaceutical scinces,* 2007 Vol. 69 (2) Page : 202-205.
14. Deshpande P B, Shridharan G, Anandi Libi, Jadhav D, Damle M C, Gandhi S V; Validated Method Development for Estimation of Atorvastatin Calcium and Fenofibrate in Fixed Dose Combination by HPTLC, Kongposh publications *THE PHARMA REVIEW* (MAY 2009)
15. Sonawane S S, Shirkhedkar A A, Fursule R A, Surana S J; Simultaneous spectrophotometric estimation of atorvastatin calcium and ezetimibe in tablets. *Indian journal of pharmaceutical scinces*, 2007 vol 69 (5) Page : 683-684.
16. Khan M R, Jain D; Simultaneous spectrophotometric determination of atorvastatin calcium and amlodipine besylate in tablets, *Indian journal of pharmaceutical scinces*, 2006 Vol. 68 (4) Page : 546-548.
17. Sahu R, Patel V B; Simultaneous spectrophotometric determination of amlodipine besylate and atorvastatin calcium in binary mixture, *Indian journal of pharmaceutical scinces*, 2007 Vol. 69 (1) Page : 110-111.
18. Palled M S, Chatter M, Rajesh PMN, Bhat A R; Difference spectrophotometric determination of telmisartan in tablet dosage forms, *Indian journal of pharmaceutical scinces*, 2006 Vol. 68 (5) Page : 685-686.
19. Mishra P, Gupta A, Shah K; simultaneous estimation of atorvastatin calcium and amlodipine besylate from tablets, *Indian journal of pharmaceutical scinces*, 2007 Vol. 69 (6) Page: 831-833.
20. Thamake S L, Jadhav S D, Pishawikar S A; Development and Validation of Method for Simultaneous Estimation of Atorvastatin Calcium and Ramipril from Capsule Dosage Form by First Order Derivative Spectroscopy, *Asian J. Research Chem*. 2(1): Jan.-Mar. 2009 ISSN 0974-4169 Page no:52-53.
21. Ma L, Dong J, Chen X J and Wang G J; development and Validation of Atorvastatin by LC–ESI–MS and Application in Bioequivalence Research in Healthy Chinese Volunteers, *Chromatographia* Volume 65, Numbers 11-12 June, 2007 ISSN0009-5893 Pages 737-741.
22. Yan T, Li H, Deng L, Guo Y, Yu W, Fawcett J. P, Zhang D, Cui Y, and Gu J; liquid chromatographic–tandem mass spectrometric method for the simultaneous quantitation of telmisartan and hydrochlorothiazide in human plasma, *Journal of Pharmaceutical and Biomedical Analysis* Volume 48 (4) 1 December 2008, Pages 1225-1229.
23. www.wikipedia.com