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Research Article

SIMULTANEOUS ESTIMATION AND VALIDATION OF ATORVASTATIN CALCIUM AND UBIDECARENONE (Coenzyme Q10) IN COMBINED TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

This paper describes a simple, precise and accurate HPLC method for simultaneous estimation of atorvastatin calcium and ubidecarenone as the bulk drug and in tablet dosage forms. The separation was achieved using a PEERLESS C_8 reverse phase column (250 x 4.6mm, 5µ, L7 pack) at ambient temperature with an isocratic mixture of methanol and acetonitrile in the ratio of 80:20 %V/V at a flow rate of 1.5mL/minute and detection at 290nm. The retention times for atrovastatin calcium and ubidecarenone were 1.692 and 10.709 minutes respectively. The linearity for atrovasatatin calcium and ubidecarenone were found to be 10 to 30 µg/mL and it obeys beers law with correlation coefficient of 1.000 for atorvastatin and 0.9991 for ubidecarenone. The proposed method was validated as per the ICH guidelines. The percentage recovery obtained for atorvastatin calcium and ubidecarenone were 99.55±09 and 101.04±11, respectively.

The method is accurate, precise and found to be suitable for the quantitative analysis of both the drugs in combinational dosage form.

Keywords: Atorvastatin Calcium, Ubidecarenone, HPLC

INTRODUCTION

Atorvastatin calcium is chemically, [R-(R*-R*)]-2-(4-fluorophenyl) –β-d-dihydroxy-5-(1-methyl ethyl) -3-phenyl-4-(phenyl amino) carbonyl)- 1H- pyrrole -1- heptonic acid, calcium salt (2.1) trihydrate. This drug is a selective, competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methyl glutaryl-coenzyme A to mevalonate, a precursor of sterols, including cholesterol[1]. Ubidecarenone is 2+-[(all-E)-3,7,11,15,19,23,27,31,35,39-decamethl tetra conta- 2,6,10,14,18,22,26,30,34,38 – decaenyl] -5,6-dimethoxy, 3-methyl benzene – 1.4 –dione, which is also called as coenzyme Q10. It is used as a dietary supplement and cardio vascular agent in congestive heart failure and angina pectoris[2]. The proposed mechanism of ubidecarenone on benefiting congestive heart failure is through positive inotropic action, which increases the contractive force of the heart to improve cardiac output. So the doses of 20-200mg/day as adjuvant therapy have been shown to be beneficial. From the literature survey, it was found that there are few analytical methods reported for atorvastatin calcium and ubidecarenone either individually [3-8] or in combination with other drugs by spectrophotometry[9] and HPLC methods[10-11]. There is not a single HPLC method has been reported for the simultaneous determination of the Atorvastatin calcium and ubidecarenone in combined dosage forms. So it was felt that there is a need to develop analytical method for the estimation of atorvastatin calcium and ubidecarenone simultaneously in a single step process. This paper presents RP-HPLC method for simultaneous determination of Atorvastatin calcium and ubidecarenone in bulk and in tablet dosage form.

MATERIALS AND METHODS

Chemicals and Reagents

HPLC grade methanol and acetonitrile (E. Merck) were used for the analysis. Water obtained form Milli-Q RO water system. Atorvastatin calcium and ubidecarenone standards used in this study were gifted by SYNTHO Lab chemicals & Research, Mumbai and NEO MEDICHEM PVT Ltd, Hyderabad, respectively. The tablets used in this study were UBICARONE, (Madras pharmaceuticals) tablets labeled to contain 10mg of Atorvastatin calcium and 10mg of ubidecarenone.

Instrumentation

Chromatographic separation was performed on a SHIMADZU chromatographic system equipped with LC-20AT pump. Variable wavelength programmable UV/Visible detector SPD-20A and Rheodyne (772 5i) with 20µL fixed loop are used and data analysis is done by using SPINCHROM software. Weighing was done on shimadzu balance (Model AY-120).

Chromatographic conditions

Separation and analysis was carried out on Peerless C_8 (250 x 4.6mm, 5µ, L7pack) column. Mobile phase consisting of a mixture of methanol and acetonitrile in the ratio of 80:20% V/V was delivered at flow rate of 1.5mL/minute with detection at 290nm. The mobile phase was filtered through a 0.45 µm membrane filter and sonicated for 15 minutes. Analysis was performed at ambient temperature.

Preparation of standard solution

Accurately weighed 10mg of atorvastatin calcium and 10 mg of ubidecarenone were transferred into a 100mL volumetric flask, then 3mL of ether was added and shaken well to dissolve and sonicated for 5 minutes, volume was made up to 100mL with methanol. The solution was further diluted with methanol to achieve final concentration of 10µg/mL of each drug and filtered through a 0.45µm membrane filter before injection.

Sample preparation and assay

A pharmaceutical sample containing atorvastatin calcium and ubidecarenone, equivalent to each 10mg, was weighed and transferred to 100mL volumetric flask. The contents of the flask were dispersed in 3mL of ether and 70mL of methanol and shaken well to dissolve and sonicated for 30minutes. Finally, the volume was made up to 100 mL with methanol and further dilution was made to get 10µg/mL concentration of each drug. The solution was filtered through 0.45 µm membrane filter before injection. All determinations were conducted in triplicate. Both the standard and sample preparation was injected separately, and the peak area responses were recorded. The percentage label claim was calculated and given in table-1.

Table 1: Assay of tablets

Drug name	Label Claim mg/tab	Mean Peak Area		Amount found* ± SD (mg/tab)	%Label claim ± SD
		Standard	Sample		
Atorvastatin calcium	10 mg	3093635	3174696	9.97±0.02	99.7 ±0.01
Ubidecarenone	10 mg	1050865	1078905	10.05±0.05	100.56±0.05

RESULTS AND DISCUSSION

The simultaneous estimation of atorvastatin calcium and ubidecarenone in tablet for was carried out by RP-HPLC using methanol and acetonitrile as mobile phase in the ratio of 80:20% v/v and Peerless C8 column as the stationary phase. The results of system suitability parameters such as tailing factor, asymmetry and number of theoretical plates are indicated satisfactory results and tabulated in table-2. The retention time for atorvastatin calcium and ubidecarenone were found to be around 1.69 and 10.70 minutes. The resolution value of more than 2 indicates satisfactory results in quantitative work and the high resolution value obtained indicate the complete separation of the drugs. The linearity was studied in the concentration range from 2-20µg/mL for atorvastatin calcium and ubidecarenone. The regression co-efficient (R^2) value for Atorvastatin calcium and ubidecarenone were found to be 1.0 and 0.9991, respectively. The mean recovery for Atorvastatin calcium was 99.0 to 100.0% and 100.2 to 101.51% for ubidecarenone, which is largely within the 90-110% range that is considered acceptable and it revels that the method is accurate.

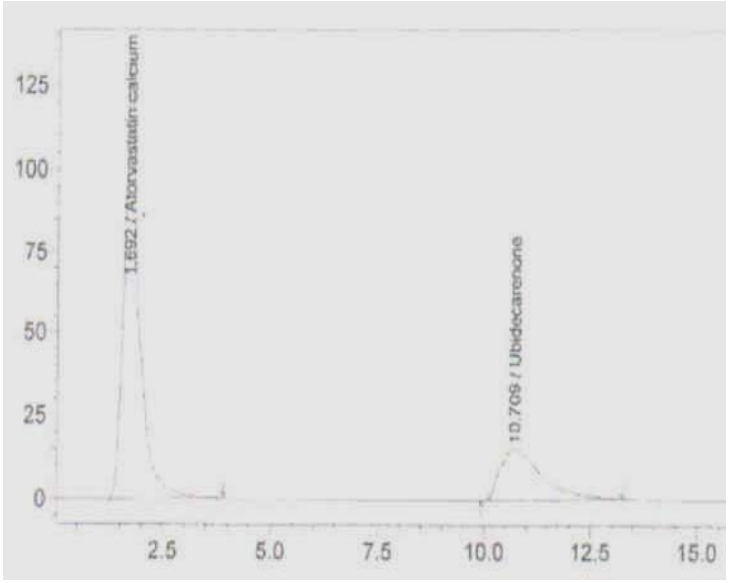

Fig. 1: Chromatogram of standard solution

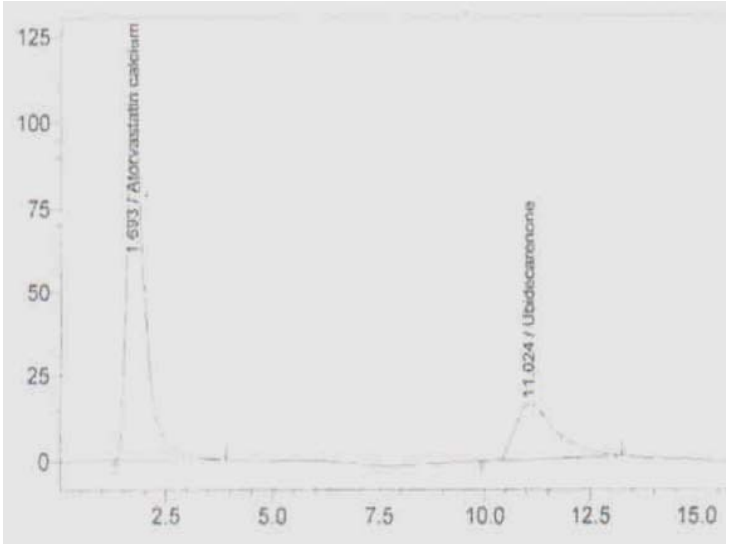

Fig. 2: Chromatogram of sample

The validation of the proposed method was verified by system precision and method precision. The system precision was evaluated by measuring the peak area responses of Atorvastatin calcium and ubidecarenone for five replicate injections of the standard solutions. The method precision was determined by quantifying the sample solutions as per the proposed method. The %RSD was found to be less than 2 for both drug indicates the proposed method is precise. The specificity of the method was confirmed by injecting the placebo and observed that there was no interference due to placebo. Robustness of the method is determined by analyzing the sample in duplicate with varying the method conditions i.e., very small changes in flow rate, showed there were no marked changes in chromatographic behavior and content of the drug, as evident from the low value of RSD indicating the method is robust. The method was also confirmed by ruggedness study, analyzing the product day to day, analyst to analyst and instrument to instrument.

The data for ruggedness of atorvastatin calcium and ubidecarenone are found to be within the acceptance limit. Different validation parameters for the proposed HPLC method for determining atorvastatin calcium and ubidecarenone were summarized in table-3 and chromatogram of atorvastatin calcium and ubidecarenone after separation is shown in fig-1 and fig-2. The result obtained was in agreement with the labeled value of atorvastatin calcium and ubidecarenone in dosage form. The determined validation parameters are in the acceptable ranges.

Table 2: System suitability parameters

S. No.	Parameters	Obtained values	
		Atorvastatin calcium	Ubidecarenone
1.	Theoretical plates (N)	5868	3233
2.	Tailing factor (T)	1.06	1.01
3.	Asymmetry	1.16	1.18
4.	% RSD of Peak Retention Time	0.156%	0.104%

Table 3: Summary of validation parameters

Parameters	Data	
	Atorvastatin calcium	Ubidecarenone
Linearity range	2 – 20µg/mL	2 - 20µg/mL
Correlation coefficient	1.000	0.9991
Limit of detection	5 ng/mL	15ng/mL
Limit of quantitation	15ng/mL	40ng/mL
%Recovery (n=6)	99.0 to 100.0%	100.2 to 101.51%
Precision (%RSD)		
System Precision	0.699%	1.090%
Method precision	0.375%	0.338%
Robustness (%RSD)	0.747%	0.394%
Ruggedness (%RSD)	0.773	0.624

CONCLUSION

The proposed method is simple, accurate, cost effective, less time consuming and the statistical analysis proved that the method is reproducible and efficient for the simultaneous estimation of Atorvastatin calcium and Ubidecarenone as bulk drugs and in combined pharmaceutical dosage forms without any interference from the excipients.

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