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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ATORVASTATIN CALCIUM AND UBIDECARENONE TABLET BY RP-HPLC

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ABSTRACT

A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of atorvastatin calcium and ubidecarenone in tablet formulation. The separation was achieved by 250x4.6 mm, 5µ, L-7 pack (peerless C-8) column and Methanol : Acetonitrile (80:20) as mobile phase, at a flow rate of 1.5 ml/min. The objective of this validation of an analytical procedure is to demonstrate that the drug Atorvastatin calcium and Ubidecarenone is suitable for its intended purpose. Developed method was found to be accurate, precise, selective, economical and rapid for simultaneous estimation of atorvastatin calcium and ubidecarenone in tablets.

INTRODUCTION: Atorvastatin ([R-(R*-R*)]-2-(4-fluorophenyl)- β, δ– dihydroxy-5- (1-methylethyl)-3-phenyl- 4 (phenylamino) carbonyl)- 1H- pyrrole- 1-heptanoic acid) is a selective, competitive inhibitor of HMG-CoA reductase and used as Synthetic lipid-lowering agent [1,2]. Ubidecarenone (2+-[(all-E)-3, 7, 11, 15, 19, 23, 27, 31, 35, 39-Decamethyltetraconta-2, 6, 10, 14, 18, 22, 26, 30, 34, 38-decaenyl]-5, 6-dimethoxy-3-methylbenzene-1, 4-dione.) is a dietary supplement and used as heart diseases, gum disease and immune deficiencies [3].

Combination of both is very useful in heart diseases. Atorvastatin calcium is previously also estimated by RP-HPLC but in different drug combinations like fenofibrate, ramipril [4], amilodipine [5], aspirin etc. now we can also estimate atorvastatin calcium with combination of ubidecarenone in tablet dosage form.

Method validation has received considerable attention in the literature and from industrial committees and regulatory agencies. The U.S. FDA CGMP (1) request in section 211.165 (e) methods to be validated: The accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented.

Such validation and documentation may be accomplished in accordance with Sec. 211.194(a). These requirements include a statement of each method used in testing the sample to meet proper standards of accuracy and reliability, as applied to the tested product.

The USP has published specific guidelines for method validation for compound evaluation. USP defines eight steps for validation: Accuracy, Precision, Specificity, Limit of detection, Limit of quantitation, Linearity and range, Ruggedness, Robustness.

Experimental Methodology:

Chromatographic Parameters:

- Instruments**:** HPLC-AGILENT 1100 series with U.V. detector and HPLC- SHIMANDZU PROMINENCE with PDA detector.
- Column**:** 250x4.6 mm, 5µ, L-7 pack (peerless C-8) or equivalent.
- Flow rate**:** 1.5 ml/minute.
- Detection wavelength**:** 290 nm.
- Injection volume**:** 20 µl.
- Column temperature**:** 30°C
- Mobile phase **:** Filtered and degassed the mixture of Methanol : Acetonitrile (80:20) [6].

Preparation of standard solution: Weigh accurately about 20.00mg of Atorvastatin calcium and 20.00mg of Ubidecarenone working standard in a 100ml volumetric flask. Add 3.00ml of ether, shake well to dissolve and sonicate for 3.0 minutes. Make the volume to 100.00ml with Methanol [7, 8, 9].

Preparation of test solution: Weigh accurately about 240.0mg of tablet powder in a 100 ml volumetric flask, add 3.0ml of ether, shake well to dissolve and sonicate for 3.0 minutes. Add 70.0 ml of methanol and shake well to dissolve and sonicate for 30.0 minutes. Make up the volume to 100.0 ml with methanol, filtered. Use this filtrate as test solution [10, 11, 12, 13].

RESULT AND DISSCUSSION: The individual peaks of Atorvastatin calcium and Ubidecarenone were identified by knowing their retention times, which were found to be around 1.692 and 10.709. Linearity was evaluated by visual inspection of plot of peak area as a function of analyte concentration for both Atorvastatin calcium and Ubidecarenone. The retention value of more than 2 indicates satisfactory results in quantitative work and the high resolution value obtained indicates the complete separation of drugs. The RSD values obtained are below 2% indicating the precision of applied methodology (**Table 1, 4**). The percentage recovery (Table 4) was found to be within 99 to 100% w/w for Atorvastatin calcium and 100.2 to 101.5% w/w for Ubidecarenone, this serves as a good index of accuracy (**Table 2, 3**) and reproducibility of the proposed method [14, 15].

The specificity of the method was confirmed by injecting the placebo and placebo spiked standard and observed that there was no interface due to placebo. This confirms the specificity of the proposed method (Table 4 and **Figure 1, 2, 3 & 4**). The validation of the proposed method was further verified by precision studies. This was done by system precision and method precision and the percentage RSD values for both system and method was found to be within the limit (Table 4). All the parameters including Flow rate, Detection, Wavelength and Sensitivity were maintained constant throughout the procedure [16, 17].

TABLE 1: STATISTICAL DATA OF ASSAY

Sample	Assay (% w/w)	Standard Deviation (S.D.)	Relative Standard Deviation (% R.S.D.
Atorvastatin Calcium	99.726	0.374	0.375
Ubidecarenone	100.438	0.399	0.338

* Each value is a mean of five readings. Acceptance Criteria: RSD NMT 2.0

TABLE 2: ACCURACY STUDY OF ATORVASTATIN CALCIUM

Target conc. (%)	Theoretical Value (mg)	Practical value (mg)	% Recovery
50	200.2	198.49	99.15
50	200.2	199.99	99.9
50	200.2	199.79	99.8
100	200.2	199.52	99.66
100	200.2	198.59	99.2
100	200.2	198.19	99.0
150	200.2	199.9	99.85
150	200.2	198.8	99.3
150	200.2	200.2	100.0

TABLE 3: ACCURACY STUDY OF UBIDECARENONE

Target conc. (%)	Theoretical Value (mg)	Practical value (mg)	% Recovery
50	200.2	203.22	101.51
50	200.2	201.6	100.7
50	200.2	201.4	100.6
100	200.2	202.46	101.13
100	200.2	200.6	100.2
100	200.2	201.8	100.8
150	200.2	201.16	100.48
150	200.2	201.8	100.8
150	200.2	202.0	100.9

TABLE 4: SUMMARY OF VALIDATION DATA

Name of analyte	Ubistatin	
Method of reference	**In-House**	
Charateristics	**Acceptance Criteria**	**Observations**
Accuracy Percent of recovery of Atorvastatin calcium Ubidecarenone	98-102%	99.0 to 100.0% 100.2 to 101.51%
System Precision Atorvastatin calcium Ubidecarenone	RSD: Not more than 2.00%	0.699% 1.09%
Method Precision Atorvastatin calcium Ubidecarenone	RSD: Not more than 2.00%	0.375% 0.338%
Specificity	No interference by placebo	Complies
Linearity Atorvastatin calcium ubidecarenone	Regression coefficient: Not less than 0.995	1.0000 0.9981
Ruggedness Atorvastatin calcium Ubidecarenone	RSD: Not more than 2.00%	0.773% 0.624%
Robustness Atorvastatin calcium Ubidecarenon	RSD: Not more than 2.00%	0.747% 0.394%

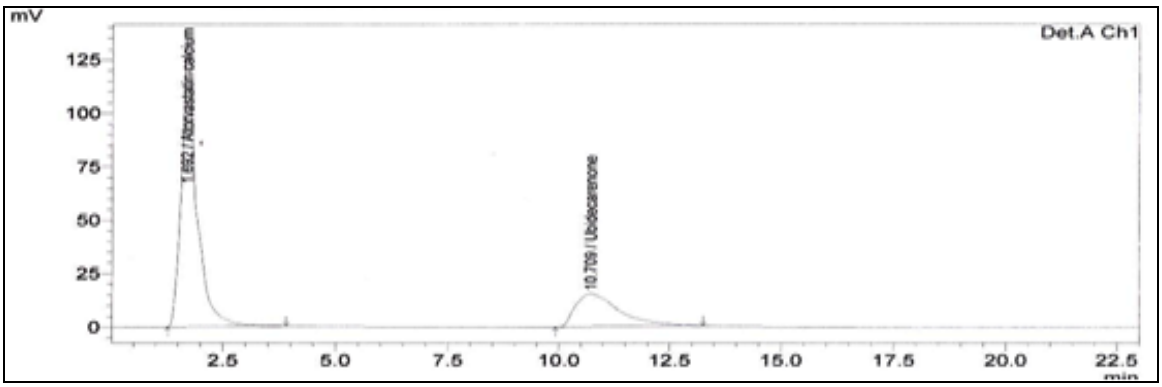

FIG. 1: SPECIFICITY CONCENTRATION (STANDARD) CHROMATOGRAM

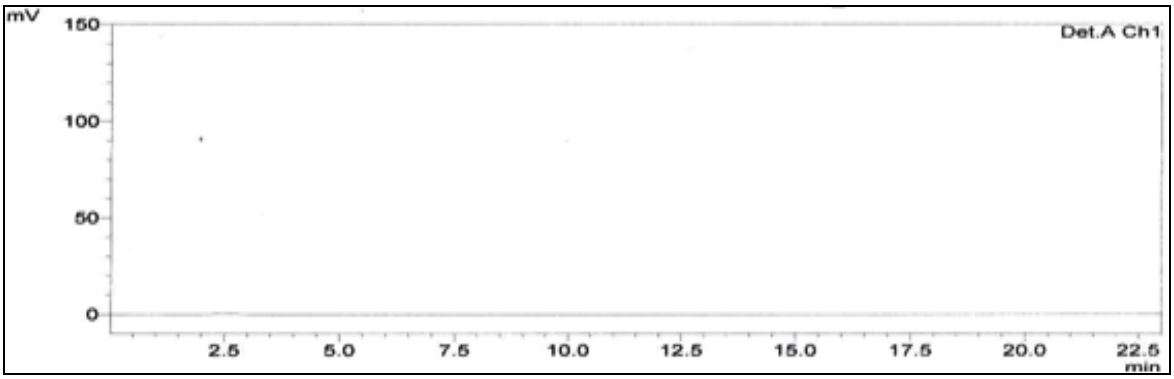

FIG. 2: SPECIFICITY CONCENTRATION-I (BLANK) CHROMATOGRAM

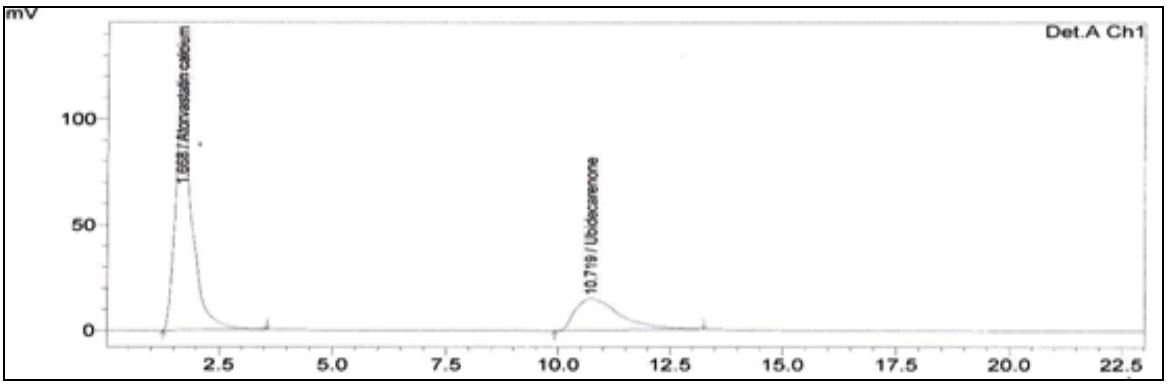

FIG. 3: SPECIFICITY CONCENTRATION-II (STANDARD) CHROMATOGRAM

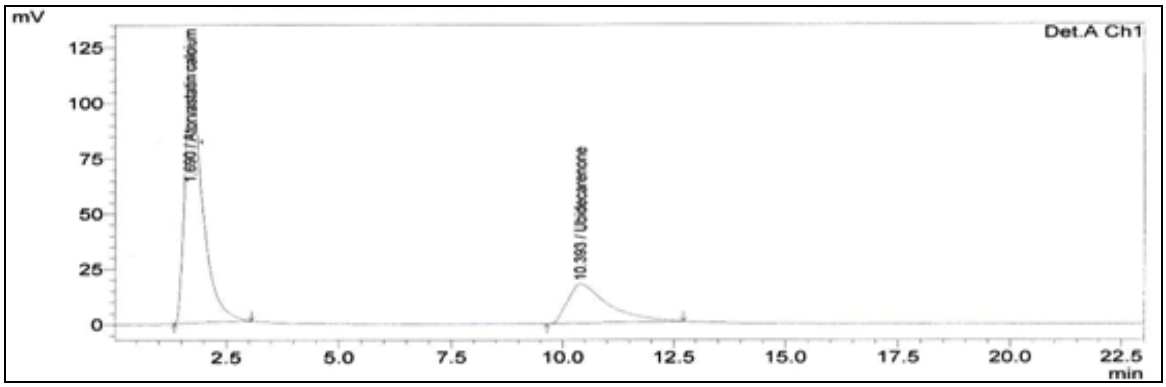

FIG. 4: SPECIFICITY CONCENTRATION-III (STANDARD+PLACEBO) CHROMATOGRAM

CONCLUSION: The retention time for Atorvastatin calcium and Ubidecarenone were found to be around 1.692 and 10.709 minutes respectively of standards. Method developed for simultaneous determination of Atorvastatin calcium and Ubidecarenone in tablet dosage form were rapid, sensitive, reproducible and economical. The RP-HPLC method so developed was simple and does not suffer from common excipients present in pharmaceutical preparation. The method was found to be highly useful in the analysis of drugs in pharmaceutical preparation.

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