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RP-HPLC Method Development and Validation For the For the Estimation of Atorvastatin Calcuim

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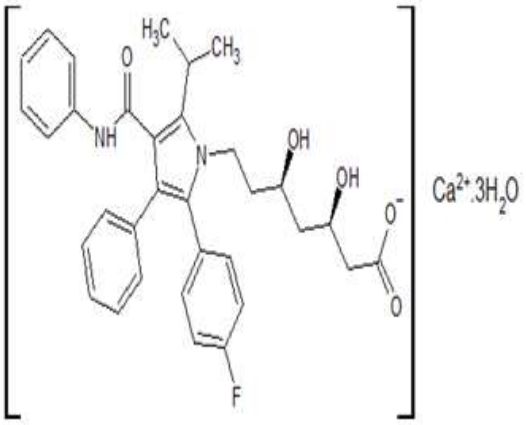
ABSTRACT: For the purpose of determining the Atorvastatin calcium, simple, precise, and accurate reverse phase highperformance liquid chromatography method was developed, Using a Phenomenex RP-C18 column (250 X 4.6 mm, 5 µm). The analysis was carried out using a methanol: water (90:10) as mobile phase, at a flow rate of 1 ml/min and detected at 254 nm.It was found that Atorvastatin had a retention time was found to be 3.81 min. The method was validated according to ICH guide lines. Linearity of method was found to be 100-250µg/ml. Percentage RSD in accuracy, intraday and interday precision were found to be 0.5244%, 0.658% and 0.4428 respectively.

KEY WORDS: Atorvastatin calcium, RP-HPLC, validation, Method development, validation

I. INTRODUCTION

Atorvastatin is chemically (3R,5R)-7-[2-(4-fluorophenyl) -3-phenyl-4-(phenylcarbamoyl)-5-(propan-2yl)1H-pyrrol-1-yl-3,5-dihydroxyheptanoic acid calcium salt trihydrate .The hepatic enzyme HMG-CoA reductase is selectively and competitively inhibited by atorvastatin. [1].Atorvastatin calcium is a white, crystalline powder with a molecular weight of 1209.41 g/mol.[2].It has been shown to be effective in lowering cholesterol and triglycerides. [3Atorvastatin calcium is often administered at a dose of 10–80 mg per day, and it lowers LDL by 40–60%.[4].Atorvastatin calcium is highly soluble in acetonitrile, distilled water, and phosphate buffer; it is also easily soluble in methanol; it is very slightly soluble in ethanol; and it is insoluble in pH≤4solutions[5].

Atorvastatin has a 14% oral bioavailability, it undergoes extensive first-pass metabolism both in the gut wall and in the liver. A total of 381 litres of atorvastatin acid are distributed, and plasma protein binding is greater than 98%. Atorvastatin acid undergoes substantial oxidation, lactonization, and glucuronidation in the gut and liver. The metabolites are then removed through biliary secretion and direct blood-to-intestine secretion. [6].



Atorvastatin Calcium

From the literature review, the atorvastatin shows the larger retention time**.** So, we aims to develop and validate RP-HPLC method for the estimation of Atorvastatin calcium.

II. MATERIALS

Chemicals and reagents

Atorvastatin calcium pure procured from Kerala state drugs and pharmaceuticals Ltd, Alappuzha, Kerala. Methanol and water were of HPLC grade. HPLC grade Methanol were obtained from Merck life science (Mumbai, India) and water obtained from Research- lab fine chem industries (Mumbai, India).

Instruments

Shimadzu HPLC model , LC-20AD pump, detector SPD-20, LC soft ware with Phenomenex RP- C_{18}(250X 4.6mm,5µm) column were used. All weights were taken on electronic balance (INFRA DIGI).

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III. EXPERIMENTATION

Preparation Atorvastatin calcium solution 1000µg/ml sstock solution of atorvastatin calcium was prepared by dissolving working standard in methanol and diluting with the same solvent .10µg/mlconcentration of atorvastatin calcium was prepared by diluting the stock solution.

The Atorvastatin calcium solution20µLwas injected to Phenomenex RP-C_{18} (250X 4.6mm,5µm) column. Methanol: water (90:10) as a mobile phase at a flow rate 1ml/min and detected at 254nm.

Method validation

The method validated for accuracy, precision, linearity and range, LOD,LOQ, robustnessand ruggedness.

IV. RESULT AND DISCUSSION

Method optimization

For HPLC method optimization different ratios of methanol and water were tried. It was found that methanol: water in the ratio of 90:10 at flow rate 1mL/min gave a good peak. Hence different combination of mobile phases and chromatographic conditions was tried, methanol: water (90:100) as the mobile phase, Phenomenex RP-C_{18} (250X 4.6mm,5µm), 1mL/min flow rate, 20µL injection volume, at ambient temperature, 254nm wave length was found to be suitable for HPLC method for standard atorvastatin calcium.

Chromatogram of Atorvastatin

Retention time was found to be 3.81min.

Method validation

Linearity and range

The concentrations range from 50, 100, 150, 200, 250 µg/ml of standard Atorvastatin calcium. Triplicate dilutions of each of the above mentioned concentrations were prepared separately and from these triplicate solutions, 20µL of each concentration of the drug was injected to the HPLC system two times separately. The linearity was found in the range of 100-250µg/ml.

Precision

Demonstrated by intraday precision and interday precision. The % RSD of intraday precision and interday precision was found to be 0.658 and 0.4428% respectively.

Accuracy

Accuracy of the method was evaluated by recovery study. The % RSD was found to be 0.5244%.

Limit of detection(LOD)

LOD=3.3σ/s

Where, σ - Standard deviation and s-Slope of the calibration curve.

The LOD was found to be 2.5µg/ml

Limit of quantification (LOQ)

LOQ=10σ/s

Where, σ- Standard deviation and s- Slope of the calibration curve.

Robustness

Robustness of the method was done by analysing six replicates of solutions. The% RSD of robustness was found to be 0.167%.

Ruggedness

Ruggedness is the reproducibility of the test result obtained by analysing the sample under varityogf conditions. % RSD of analyst 1 was found to be 0.2988% and analyst 2 % RSD was found to be 0.4000%.

LINEARITY AND RANGE	100-250 µg/ml
INTRADAY PRECISION	%RSD 0.658 WITH IN THE LIMIT 2%
INTERDAY PRECISION	%RSD 0.4428 WITH IN THE LIMIT 2%
ACCURACY	%RSD 0.5244 WITH IN THE LIMIT 2%
LOD	2.5 µg/ml
LOQ	10 µg/ml
ROBUSTNESS	%RSD 0.167 WITH IN THE LIMIT 2%
RUGGEDNESS	ANALYST 1: %RSD 0.2988 WITH IN THE LIMIT 2% ANALYST 2: %RSD 0.400WITH IN THE LIMIT 2%

Method validation

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V. CONCLUSION

The RP-HPLC method developed for the determination of standard Atorvastatin calcium is simple, precise, and accurate with short run time. The method was fully validated for showing satisfactory data for all the method validation parameters are test. The developed method are conveniently used for the determination of standard Atorvastatin calcium.

REFERENCES

[1]. Gennaro, A.E., 2000, 21 Ed.. In: Remington's The Science and Practice ofPharmacy, vol. II Mack Publishing Co, Easton, PA, 1294, 1368, 1705.
[2]. Al Shehri, M.M. (2012) Saudi Pharm. J. 20, 143–148.
[3]. Poswar, E. L., Radulovic, L. L., Cilla, D. D., Whitfield, L. R. and Sedman, A. J., Tolerance and pharmacokinetics of single-dose atorvastatin, a potent inhibitor of HMG-CoA reductase,in healthy subjects (1996) J. Clin. Pharmacol. , 36: 728.
[4]. Page, Curtis, Sutter, Walkar and Hoffmann (2002) Integrated Pharmacology 2nd, Mosby International Ltd., p 303.
[5]. O'Neil, M.J.(2013) The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals, 15Ed.,Merck, Whitehouse Station,NJ
[6]. Hans Lennernas. Clinical pharmaco kinetics of atorvastatin.Clin Pharmacokinet 2003; 42 (13): 1141-1160.