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SHORT COMMUNICATION article

Chiral screening approach of atorvastatin diastereomers by HPLC method

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Abstract: The current study seeks to develop and validate a high-performance liquid chromatography method for atorvastatin diastereomer separation and analysis. In particular, we wish to identify the many diastereomers in atorvastatin, which can help us to better understand their pharmacological properties and provide significant information for pharmaceutical applications. Atorvastatin was chromatographed on a Chiralcel® OD-RH column and n-hexan-2-propanol (95:05 v/v) as the mobile phase, with an injection volume of 10 µL. The solution was pumped at a continuous flow rate of 1 mL/min, with a detection wavelength of 260 nm. The investigation found two peaks with retention times of 3.23 and 3.85 min, respectively. The resolution, capacity, and selectivity factors obtained were Rs = 1.2, k′1 = 3.50, k′2 = 4.37, and α = 1.24.

Introduction

Most pharmaceutical companies are increasingly focusing on the therapeutic effects of diastereomers of existing pharmacological compounds to develop a detailed impurity profile [1]. Diastereomers are stereoisomers that are not related to objects and mirror images and are not enantiomers. Unlike enantiomers which are mirror images of each other and non-superimposable, diastereomers are not mirror images of each other and non-superimposable. Diastereomers can have different physical properties and reactivity. They have different melting points, boiling points and different densities**.** Hyperlipidemia, or an increase in lipid concentration in the blood, is a symptom indicating a problem with the synthesis and breakdown of plasma lipoproteins. Cholesterol and triglycerides are the major lipids involved in hyperlipidemia. One of the seven frequently and mostly used statins to lower blood cholesterol and for prevention of events associated with cardiovascular disease called atorvastatin which is a synthetic lipid-reducing drug and a 3-hydroxy-3-methyl-glutaryl inhibitor, it catalyzes the conversion of HMG-CoA to mevalonate which is an important rate-limiting step in cholesterol biosynthesis [2-7]. Atorvastatin belongs to a group of medicines called statins. It is used to lower cholesterol if you've been diagnosed with high blood cholesterol and prevent heart disease, including heart attacks and strokes. It is chemically named as (3R, 5R)-7-(2-[4-fluorophenyl]- 3-phenyl-4-[phenyl carbamoyl]-5-propan-2-ylpyrrol-1-yl)-3,5 dihydroxyheptanoic acid (**Figure 1**). Structurally, atorvastatin is a chiral compound that has two asymmetric centers in the molecule, which allows it to produce four different enantiomers: 3R5R, 3R5S, 3S5R and 3S5S [8-13]. The importance of chiral separation of atorvastatin stems from the fact it is a chiral molecule that exists as two diastereomers, (S, S) and (R, R). Because these

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enantiomers have different pharmacological properties, chiral separation of enantiomers is especially important in drug development because one enantiomer may be responsible for the therapeutic effect, while the other may cause unwanted side effects. In the case of atorvastatin, (S, S) enantiomer is the active form of the drug, whereas the (R, R) enantiomer is inactive [1, 14, 15]. In the analysis of most pharmaceutical formulations and biological materials, High-Performance Liquid Chromatography (HPLC) is the technique of choice. Several detailed literature revealed that many methods have been reported for the analysis of atorvastatin either in bulk powder, different dosage forms or in biological fluids individually or as a combination with other drugs using a different stationery phase most of them used C8 [16,17], C18 columns [2-8, 11-13, 18-20, 21] to confirm the purity of the compound analyzed, and they used other columns such as chiral pack@ AD-H [1] and phenyl [10] columns to determine the enantiomers of this chiral molecule. The current study aimed at developing a simple and precise HPLC method for detecting atorvastatin diastereomers utilizing the Chiral®cel OD-RH column (**Figure 2**).

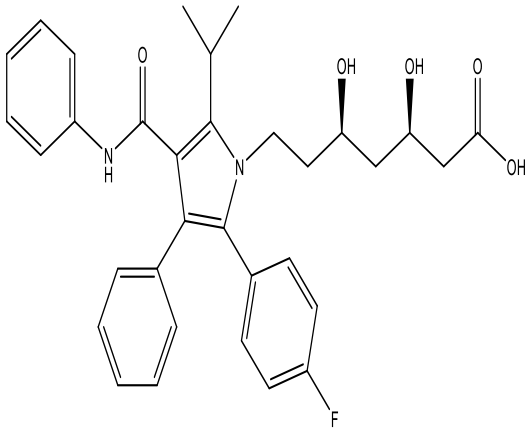

Figure 1: Chemical Structure of atorvastatin

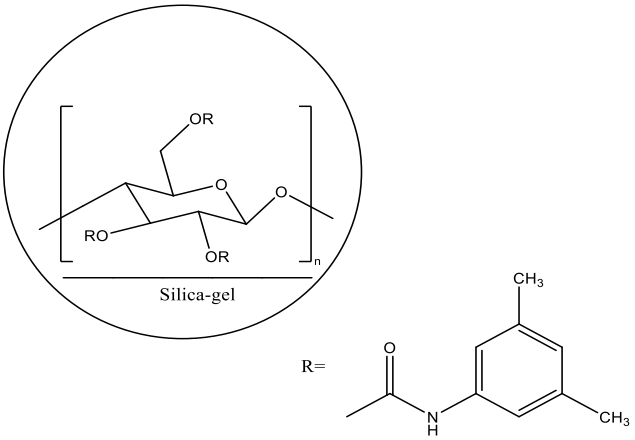

Figure 2: Structure of Chiral cel® OD-RH column

Materials and methods

HPLC-analysis: The experiments consisted SHIMADZU LC 20-A system equipped with a DGU degasser, Shimadzu® LC 20 AD LC pump, a Rheodyne injector with 20 µL Rheodyne 1907 sample loop equipped with a UV detector Shimadzu SPD-20 A (Kyoto, Japan).

Stationery and mobile phases: The chiral analysis by HPLC was carried out by using two analytical columns namely: Shim-pack GIS 5 µm C-18, 4,0×250 mm and Chiral® cel OD-RH a cellulose tris (3,5-dimethylphenyl carbamate) coated on 5 µm silica-gel column and the HPLC solvents used were, Chromasolv® methanol, Chromasolv® acetonitrile, water, LiChrosolv® n-hexane, and Chromasolv® 2-propanol for HPLC which were purchased from Sigma Aldrich, United States, with known that the used atorvastatin® (40 mg) was dissolved in methanol, in order to complete the separation in a reasonable amount of time and with an appropriate peak form.

Chromatographic conditions: The chromatographic separations were conducted at room temperature and the UV detector was set at 260 nm. Starting by the purity of the compound was checked by using a chiral C-18 column as a stationary phase and the mobile phase used was the mixture of (methanol: acetonitrile: water) in the ratio of (76:13:11) (v/v/v). The injection volume was 20 µL and the flow rate was kept constant at 1 mL/min. To accomplish the chiral separations and obtain all possible enantiomers of this chiral compound one CSPs which is Chiralcel® OD-RH was then employed, the injection volume was 10 µL under 1 mL/min flow rate and the mobile phase used was n-hexane and 2-propanol (95:5, v/v).

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Results

A C18 column's separation mechanism is based on the partitioning of analytes between the stationary and mobile phases. Our investigation found that preliminary separation with a C18-column validated the purity of atorvastatin (AT), with the shortest retention time of 1.452 minutes, which is the amount of time it takes for that component to pass through the column and elute from it. This compound has two chiral centers so four diastereomers are expected to be resolved, using the Chiralcel® OD- RH column. However, the retention times, resolution, selectivity and separation factors were good as shown in **Table 1** and **Figure 3**.

Table 1: Chiral separation of atorvastatin in Chiralcel® OD- RH column

	Peak	Rt	K′	α	Rs	Area%
Chiralcel® OD- RH	1	3.23	3.509	1.24	1.2	14.46
	2	3.85	4.374			85.54

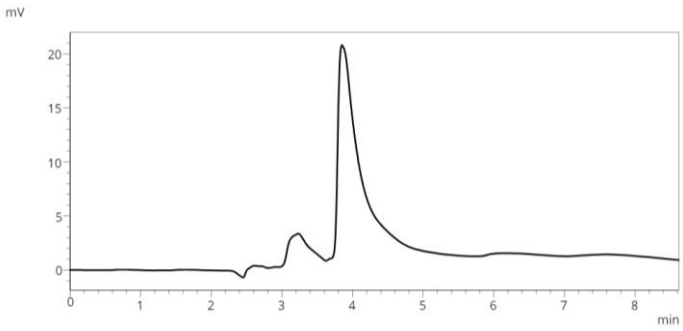

Figure 3: Chromatogram of the separation of atorvastatin by HPLC on the Chiralcel® OD- RH column, ambient temperature; flow rate 1 ml/min and wavelength at 260 nm

Discussion

The compound studied was separated under the conditions described in the experimental part. These results were also achieved after varying various HPLC parameters, such as the injection volume and flow rate. The factors capacities obtained were k′1 = 3.50 and k′2 = 4.37, respectively, with a short analysis time of 3.231 and 3.851. This method gives good resolution between both compounds (Rs = 1, 2). In chromatography, resolution refers to the degree of separation between two neighboring peaks on a chromatogram. It is critical to determine the quality and efficiency of a chromatographic separation process [22]. In this study, we obtained an acceptable selectivity factor (α1 = 1, 24). The selectivity factor (α) measures the separation between two analytes in HPLC. It is defined as the ratio between the retention factors (k) of two neighboring peaks in the chromatogram: α = k′2/k′1. The selectivity factor indicates the HPLC system's capacity to distinguish between two analytes based on their chemical and physical properties [23]. The proposed method was found to be simple and sensitive for the determination of atorvastatin in a tablet formulation.

Conclusion: A HPLC method was developed using a Chiralcel® OD- RH column which is the polysaccharide-based chiral stationary phase. The separation was successfully performed using 2-propanol, and hexane as the mobile phase. The results demonstrate clearly that a good separation was achieved with a resolution of 1, 2. The precision values were within the acceptable range, indicating that the technique was precise. Hence, the method is and can be used for atorvastatin regular analysis in pharmaceutical and bulk samples and quality control laboratories and chiral structures have a profound effect on several of biological and chemical reactions [24, 25].

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Author contribution: NB conceived & designed the study, TH collected data.TH, KS & MG contributed to the data analysis. TH & NB interpreted the data and drafted the manuscript. All the authors approved the final version of the manuscript and agreed to be accountable for its contents.
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Ethical issues: Including plagiarism, informed consent, data fabrication or falsification, and double publication or submission have completely been observed by authors.
Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.