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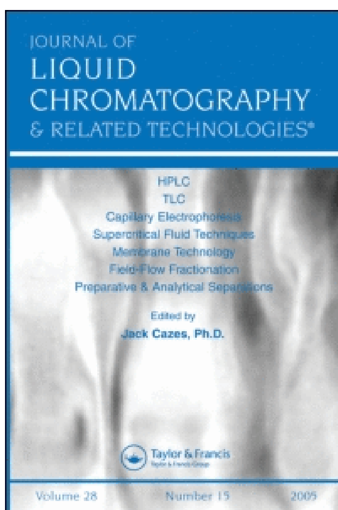

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Liquid Chromatographic Determination of Atorvastatin in Bulk Drug, Tablets, and Human Plasma

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ABSTRACT

A simple, specific, and accurate high performance liquid chromatographic (HPLC) method for determination of atorvastatin in bulk drug, tablets, and human plasma have been developed. Liquid chromatography was performed on a RP-Supelcosil C_{18} (5 μm, 150 × 4.6 mm) column and the mobile phase consisted of an acetonitrile : methanol : water (45 : 45 : 10 v/v/v), and a flow rate of 1.0 mL/min. The effluent was monitored on a UV detector at 240 nm. Each analysis required no longer than 3.0 min. Quantification was achieved by the measurement of the peak area ratio of the drug to the internal standard (ibuprofen). For quantification, a calibration curve was constructed for atorvastatin

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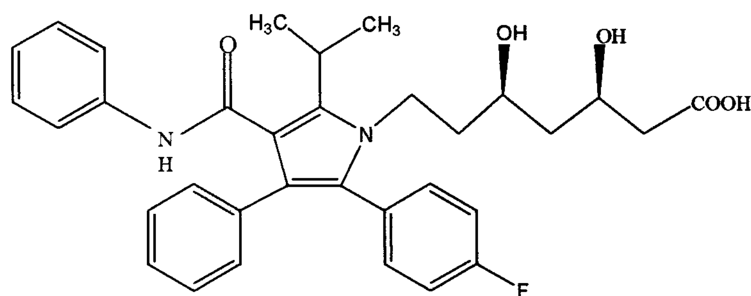
concentration ranging between 0.5–86.0 μg/mL. Furthermore, the typical excipients included in the drug formulation (starch, lactose, glucose, sugar, talc, sodium chloride, titanium dioxide, and magnesium stearate) do not interfere with the selectivity of the method. Data, with respect to precision and accuracy and limits of detection, are reported and discussed. The proposed chromatographic method was successfully applied to the quantitative determination of atorvastatin in bulk drug, tablets, and spiked human plasma.

Key Words: Atorvastatin; Ibuprofen; High-performance liquid chromatography; Bulk drug; Tablets; Human plasma.

INTRODUCTION

Atorvastatin, (βR,δR)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid calcium salt is a second generation 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, recently approved for clinical use as a cholesterol lowering agent. This synthetic HMG-CoA reductase inhibitor induces a significant reduction in total cholesterol, low-density lipoprotein cholesterol, and plasma triglycerides in clinical studies.[1,2] The chemical structure of atorvastatin is shown in Sch. 1.

Reviewing the literature revealed that all the reported methods for the determination of atorvastatin in dosage forms and biological fluids based on the use of chromatographic techniques such as LC/MS/MS, microbore LC/ESI-MS/MS, and HPLC with electrospray tandem mass spectrometry.[3–9] However, no simple LC methods with UV detector were reported for the quantitative determination of atorvastatin in bulk drug, tablets and human plasma.



Scheme 1. Atorvastatin.

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Moreover, an official method for atorvastatin determination has not yet been described in any pharmacopoeia. In the present study, a new, simple, and selective HPLC method was applied to the determination of atorvastatin in bulk drug, tablets, and spiked human plasma.

EXPERIMENTAL

Chemicals and Reagents

Atorvastatin was supplied by Sanovel Pharmaceutical Co. (Istanbul, Turkey) and the internal standard (I.S), ibuprofen, was obtained from Eczacibasi Pharmaceutical Co. (Istanbul, Turkey). ATOR® (40 mg atorvastatin) tablets were purchased through a local pharmacy. HPLC grade methanol, and acetonitrile were purchased from Merck Co., Germany. Water was bidistilled, and all the other chemicals were of analytical reagent grade and were used as received. Drug-free human plasma was obtained from the hospital blood bank.

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Instrumentation and Chromatographic Conditions

The HPLC system consisted of a membrane degasser, binary solvent delivery system, a Rheodyne injector equipped with a 20 $\mu\Lambda$ sample loop, and a UV/VIS detector (1100 Series, Agilent Technologies, USA). The detection wavelength was at 240 nm, and the peak areas were integrated automatically with Windows NT based LC ChemStation Software.

The chromatographic analysis was performed at ambient temperature onto a Supelcocil C_{18} column (150 × 4.6 mm i.d, 5 μm particle size) and a mobile phase composed of acetonitrile : methanol : water (45 : 45 : 10 v/v/v). The flow rate was maintained at 1.0 mL/min.

Preparation of the Standard Solutions and Calibration Curve

Standard solutions (1.0 mg/mL) of atorvastatin and ibuprofen were prepared daily by dissolving appropriate amounts of these substances in methanol. Stored at +5°C in the dark, these solutions were shown to be stable during the period of study. Working standard solutions containing 0.5–86.0 μg/mL of atorvastatin and 40.0 μg/mL of ibuprofen (I.S.) were prepared in methanol. A volume of 20 μL of each sample was injected into the column. All measurements were repeated five times for each concentration. The

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calibration curve was constructed by plotting the peak area ratios of analyte to I.S. vs. the corresponding drug concentration.

Spiked Human Plasma Samples

Trichloroacetic acid, perchloric acid, sulphuric acid, ethanol, and acetonitrile were tested in order to precipitate human plasma proteins. Acetonitrile was found to be the best precipitant, because when this substance was used in small volumes the precipitation was successfully completed. Acetonitrile (1.0 mL) was added to human plasma (0.5 mL) containing standard solutions of atorvastatin and I.S. Addition of acetonitrile prevents atorvastatin binding to proteins and coagulate plasma proteins. The mixtures were then vortexed for 10 min. After deproteinization and centrifugation of samples for 15 min at 6000 rpm, supernatant (0.5 mL) was taken carefully and analyzed as described above. No anticoagulant was used in these proposed methods.

Analysis of Pharmaceutical Dosage Forms

The proposed methods were used to determine atorvastatin in pharmaceutical dosage form. Ten ATOR® film tablets (40.0 mg atorvastatin per tablet) (Sanovel Pharmaceutical Co.) were triturated in an agate mortar, pounded, and finally, the correct amount of powder was dissolved in methanol by stirring for 30 min. The excipient was separated by filtration and the residue washed three times with the same solvent. After filtration, an appropriate volume of the filtered solution was taken in a 100 mL flask. The appropriate amount of internal standard was added and diluted up to the mark with the mobile phase. Solutions were filtered through 0.45-μm membrane filters. Triplicate injections (20 μL) were made for each solution. The amount of atorvastatin per tablet was calculated from the linear regression equation.

Recovery Studies

To keep an additional check on the accuracy of the developed assay method, and to study the interference of formulation additives, analytical recovery experiments were performed by the standard addition method. The known amount of the pure sample solutions were added to the preanalyzed samples of each drug, including a constant level of the internal standard, and the mixtures were analyzed by the proposed method. From the total amount of

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drug found, the percentage recovery was calculated. After five repeated experiments, the recoveries were calculated.

RESULTS AND DISCUSSION

The HPLC conditions were optimized to obtain an adequate separation of the eluted compounds. Initially, various mobile phase compositions and various analytical columns were tried in attempts to separate drug and internal standard. Mobil phase and flow rate selection was based on peak parameters (height, asymmetry, tailing), baseline drift, run time, ease of preparation of mobile phase. The system, with methanol : acetonitrile : water (45 : 45 : 10 v/v/v) mobile phase, and 1.0 mL/min flow rate is quite robust. Other analytical columns have been tested, with minimal effect on the resolution of the analytes. A RP-Supelcosil C_{18} column is recommended because of its demonstrated ruggedness and reproducibility in this assay. Ibuprofen was applied as an internal standard, neutralizing the error inherent in sample injection, eliminating random errors. A typical chromatogram for atorvastatin and ibuprofen (I.S.) using RP-Supelcocil C_{18} (150 × 4.6 mm i.d., 5 μm) column at flow rate of 1.0 mL/min is shown in Fig. 1. The optimum wavelength for detection was 240 nm at which much better detector response for drugs was obtained. In Fig. 1, for the estimation of atorvastatin and ibuprofen a sharp and

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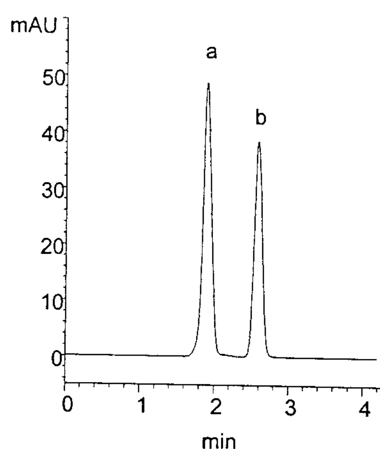

Figure 1. HPLC chromatogram of a 20 μL injection containing (a) 40.0 μg/mL of atorvastatin; and (b) 40.0 μg/mL of ibuprofen (I.S.).

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symmetrical peak was obtained with a good baseline, thus facilitating the accurate measurement of the peak area. The average retention times for atorvastatin and ibuprofen were found to be 1.9 ± 0.04, and 2.6 ± 0.01 min, respectively. Under the described HPLC parameters, the respective compounds were clearly separated and their corresponding peaks were sharply developed at reasonable retention times.

The calibration curve was linear in the range of 0.5–86.0 μg/mL. The calibration curve equation is Y: a + bC, where "Y" represents the atorvastatin peak area to ibuprofen (I.S) peak area ratio and "C" represents atorvastatin concentration. Table 1 represents calibration characteristics for the peak area ratio of varying amounts of atorvastatin to a constant amount of ibuprofen (40.0 μg/mL). Validation of the procedures for the quantitative assay of the drug were examined via evaluation of the limit of detection (LOD), limit of quantitation (LOQ), repeatability, recovery, specificity, and robustness. The LOD and LOQ were calculated from the calibration curves as $k\mathrm{SD}/b$, where $k = 3$ for LOD and 10 for LOQ, SD is the standard deviation of the intercept and b is the slope of the calibration curve. The values of LOD and LOQ were 0.0084 and 0.0179 μg/mL, respectively.

Day-to-day precision and accuracy were evaluated by using five samples of three different concentrations at low, medium, and high concentrations, which were prepared and analyzed on the same day (Table 2). Sample-to-sample variability was assessed using five samples of three different concentrations at low, medium, and high concentrations analyzed on five

Table 1. Statistical analysis of calibration curves in the HPLC determination of atorvastatin (abbreviations as in text).

Parameters	
Range (μg/mL)	0.5–86.0
Detection limits (μg/mL)	0.0084
Quantitation limits (μg/mL)	0.0179
Regression equation (Y)[a]	
Slope (b)	0.0360
Intercept (a)	0.0130
Correlation coefficient (r)	0.9980
Rel. std. dev. (%)[b]	0.8100

[a]Y = a + bC, where C is concentration in μg/mL and Y is peak area ratio.
[b]Five replicate samples.

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Table 2. Sample-to-sample (intraday) and day-to-day (interday) precision of atorvastatin standards by using high performance liquid chromatography (abbreviations as in text).

Theoretical concentration (μg/mL)	Intraday concentration (mean)	Measured[a] (μg/mL) (RSD %)	Interday concentration (mean)	Measured[b] (μg/mL) (RSD %)
0.5	0.47	0.83	0.49	1.34
40.0	40.3	0.69	40.9	1.32
86.0	85.8	0.72	85.5	0.99

[a]Mean values represent five different sample standards for each concentration.
[b]Intraday reproducibility was determined from five different runs over a week period.

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different days, over a period of a week. These results show the accuracy and reproducibility of the assay. Thus, it was concluded that there was no significant difference for the assay, which was tested on an intra-day and inter-day basis.

Specificity of the optimized procedures for the determination of atorvastatin was examined in the presence of some common excipients in the same ratios usually used in pharmaceutical preparations (starch, lactose, glucose, sugar, talc, sodium chloride, titanium dioxide, and magnesium stearate). The mean percentage recovery of 40.0 μg/mL atorvastatin showed no significant excipients interference, thus the procedures were able to assay atorvastatin in the presence of excipients and, hence, it can be considered specific. Co-administered drugs have been shown not to interfere in the assay. These include atenolol (retention time, 8.3 min), losartan (6.1 min), lisinopril (5.5 min), candesartan cilexetil (6.5 min), irbesartan (5.8 min), perindopril (3.4 min), paroxetine hydrochloride (6.6 min), olanzapine (7.5 min), fluvastatin (4.2 min), zolmitriptan (4.1 min), indomethacin (3.1 min), piroxicam (5.2 min).

Robustness was examined by evaluating the influence of small variations of some of the most important procedure variables, including pH and concentration potential. The results showed that none of these variables significantly affect the recovery of atorvastatin. These results provided an indication of the reliability of the proposed procedures for assay of the drug and could be considered robust.

To establish the validity and applicability of the proposed method, ten samples in the concentration range reported in Table 3 were assayed by the present procedures. The data shown in Table 3 indicate that the method has good accuracy and precision.

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Table 3. Recovery experiments obtained for different samples of atorvastatin by using high performance liquid chromatography (abbreviations as in text).

Amount added (μg/mL)	Amount found (μg/mL)	Recovery (%)
0.50	0.49	98.0
20.0	19.6	98.0
40.0	39.8	99.5
60.0	59.5	99.2
86.0	85.8	99.8
		$\overline{X} = 98.9$
		RSD = 0.99

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The developed method was applied to the recovery of atorvastatin in three batches of commercial formulations, respectively. The results presented in Table 4 are in good agreement with the labelled content. All data represent the average of five determinations. Low values of standard deviation indicate very good reproducibility of the measurement.

Figure 2 shows the typical chromatogram obtained from the human plasma spiked with atorvastatin, ibuprofen (I.S.), and, which indicate no interferences from the endogenous substances present in the human plasma. Human plasma samples were spiked with atorvastatin to achieve final concentrations of 0.5, 40.0, and 86.0 μg/mL. The determination results, and recoveries of known amounts of atorvastatin added to human plasma, were given in Table 5. The proposed method gives reproducible results, is easy to

Table 4. Results obtained in determination of atorvastatin in pharmaceutical dosage form[a] (abbreviations as in text).

	Mean (mg) ± SD[b]
Batch 1	40.2 ± 0.146
Batch 2	39.9 ± 0.135
Batch 3	40.7 ± 0.189

[a]Ator® film tablets were labeled to contain 40.0 mg atorvastatin, per tablet respectively.
[b]Each value is the mean of ten experiments; SD: standard deviation.

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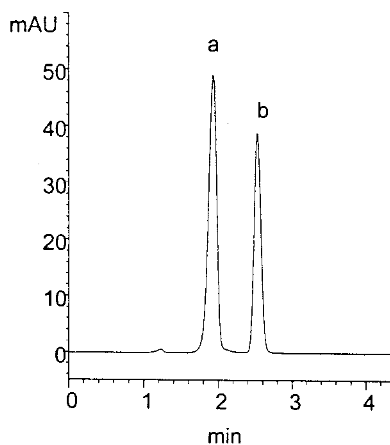

Figure 2. HPLC chromatogram of human plasma spiked with (a) 40.0 μg/mL of atorvastatin; and (b) 40.0 μg/mL of ibuprofen (I.S.).

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perform, and is sensitive enough for the determination of atorvastatin in human plasma. Care must be taken when analysing human plasma samples from patients who takes ibuprofen, since this will interfere in the analysis.

In conclusion, the proposed procedure was successfully applied to the determination of the studied compound in pharmaceutical dosage form. The proposed method gives good resolution between atorvastatin and IS with a short analysis time (<3 min). The method described is simple, rapid, and does not involve use of complex instrumentation or complicated sample preparation. A high recovery shows that the method is free from the interferences of the co-formulated excipients used in the formulations. This method could be suitable for the routine analysis of pharmaceutical

Table 5. Results obtained for atorvastatin analysis from human plasma (abbreviations as in text).

n	Added (μg/mL)	Found (μg/mL)	Recovery (%)
5	0.5	0.49	98.0
5	40.0	39.6	99.0
5	86.0	86.1	100.1
			$\overline{X} = 99.0$
			RSD = 1.0

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formulations in quality control laboratories for products of similar type and composition.

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