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An HPLC method for the determination of atorvastatin and its impurities in bulk drug and tablets

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Abstract

A simple high-performance liquid chromatographic (HPLC) method was developed for the analysis of atorvastatin (AT) and its impurities in bulk drug and tablets. This method has shown good resolution for AT, desfluoro-atorvastatin (DFAT), diastereomer-atorvastatin (DSAT), unknown impurities and formulation excipients of tablets. A gradient reverse-phase HPLC assay was used with UV detection. Some solvent systems prepared using methanol or acetonitrile and water or buffer systems with different pH values were tested. Capacity factors of related substances were calculated at all tested systems. Best resolution has been determined using a Luna C_{18} column with acetonitrile–ammonium acetate buffer pH 4-tetrahydrofuran (THF) as mobile phase. Samples were eluted gradiently with the mobile phase at flow rate 1.0 ml min^{-1} and detected at 248 nm. The proposed method was applied to the determination of impurities and were found to contain 0.057–0.081, 0.072–0.097, 0.608–0.664% of the DFAT, DSAT and total impurity, respectively.

Keywords: Atorvastatin; Desfluoro-atorvastatin; Diastreomer-atorvastatin; Impurities

1. Introduction

Atorvastatin (AT) is a synthetic hydroxy methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor that has been demonstrated to be efficacious in reducing both cholesterol and trigliceride. It is administered as the calcium salt of the active hydroxy acid and is used between 10 and 80 mg per day to reduce the raised lipid levels in patients with primary hyperlipidemia (familial and non-familial) or combine hyperlipidemia [1,2].

The drug has eight unknown and two known process impurities are called diastereomer-atorvastatin (DSAT) and desfluoro-atorvastatin (DFAT). Although some liquid chromatographic–mass spectrometric assay methods have been developed to determine AT in aqueous solutions [3,4] or in plasma [5] there is no official or analytical method appeared in the literature for resolution and determination of these impurities in the bulk drug or pharmaceutical formulations. Aim of this

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study is developing a simple, reliable determination method for AT and its impurities. Proposed method was allowed to determine AT and the purity of the bulk drug, level of impurities both in the bulk drug and pharmaceutical formulations.

2. Experimental

2.1. Material and chemicals

AT, DFAT, DSAT, unknown impurities, formulation excipients and Ator® tablets containing 10 and 20 mg AT calcium were obtained from Sanovel (Istanbul, Turkey).

Acetonitrile, methanol, tetrahydrofuran (THF) of high-performance liquid chromatographic (HPLC) grade and the chemicals, ammonium acetate, ammonium citrate, potassium dihydrogen orthophosphate, ammonia, hydrochloric acid were obtained from Merck (Darmstadt, Germany). Water was deionized and then doubly distilled.

The filters with pour size of 0.45 and 0.2 μm (Waters, Milford, USA) were used for the filtration of mobile phase and sample solution, respectively.

2.2. High-performance liquid chromatography apparatus

A P4000 HPLC pump (Thermo Separation Products Inc., Texas, USA), equipped with a Rheodyn injection system with a 100 μl loop. Detection was accomplished with an UV 3000 detector at 248 nm. Integration and system parameters were controlled by SN 4000 software system.

Chromatographic analyses were carried out at room temperature on a 5 μm C_{18} Luna column (250×4.6 mm i.d., Phenomenex) fitted with a guard column (4×3 mm i.d., Phenomenex) packed with the same material. The separations were achieved by gradient elution with a flow rate of 1.0 ml min^{-1}. Beginning ratio of mobile phase was acetonitrile–ammonium acetate buffer pH 4.0-THF 25:70:5 (v/v/v); than the ratio was changed linearly 70:25:5 (v/v/v) within 50 min and the system was continued at the same ratio for 10 min. The subsequent ratio of the mobile phase was flowed for 60 min more to be conditioned of the column.

2.3. Solutions

1.0 mg each of AT, DFAT, DSAT, and 15.0 mg each of bulk drug and tablet excipients were accurately weighed and transferred to the 10 ml volumetric flasks, separately; 1.0 ml methanol was added into the each flask and shaken for 5 min in an ultrasonic bath. The mixtures were made up to the volume with acetonitrile–water (40:60, v/v). The solutions of AT, DFAT and DSAT were mixed on a magnetic mixer at the 450 rpm for 15 min. The solutions of bulk drug and tablet excipients were mixed 60 min at the same conditions.

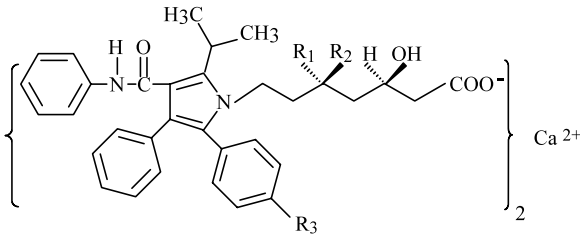
Ten coated film tablets were weighed and transferred into 100 ml calibrated flasks separately. 50 ml of acetonitrile–ammonium citrate buffer pH 4-THF (27:53:20, v/v/v) was added then the mixtures were shaken at the 450 rpm for 60 min and diluted to the volume with acetonitrile–ammonium citrate buffer pH 4-THF (27:53:20, v/v/v) and filtered. A 2 ml of each of the filtrate was diluted to 10 ml.

All of the solutions were freshly prepared and injected to the HPLC system after filtration.

Acetate buffer solution was prepared by dissolving 1.54 g ammonium acetate in 800 ml of water. The pH was adjusted to 3.0 ± 0.05, 4.0 ± 0.05, 5.0 ± 0.05 with gl acetic acid and the volume was made up to 1000 ml with water.

Citrate buffer solution was prepared by dissolving 9.62 g anhydrous citric acid in 950 ml of water. The pH was adjusted to 3.0 ± 0.05, 4.0 ± 0.05, 5.0 ± 0.05 with aqueous ammonia solutions (10%, v/v) and the volume was made up to 1000 ml with water.

Phosphate buffer solution was prepared by dissolving 13.6 g potassium dihydrogen phosphate in 900 ml of water. The pH was adjusted to 3.0 ± 0.05, 4.0 ± 0.05, 5.0 ± 0.05 with 0.1 M hydrochloric acid solution and the volume was made up to 1000 ml with water.



Atorvastatin : R_1 = OH, R_2 = H, R_3 = F
Desfluoro-atorvastatin : R_1 = OH, R_2 = H, R_3 = H
Diastreomer-atorvastatin : R_1 = H, R_2 = OH, R_3 = F

Fig. 1. Chemical structures of AT and related compounds.

3. Results and discussion

The chemical name of AT is [R-(R*,R*)]-2-(4-fluorophenyl)-B,B-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid calcium salt (Fig. 1). AT calcium is a white to off white powder is insoluble in aqueous solutions of pH 4 and below, slightly soluble in distilled water, acetonitrile and ethanol and freely soluble in methanol, chloroform and dimethyl-sulphoxide. Known and unknown impurities have similar properties with AT calcium as well.

A reverse-phase HPLC system consisting of an alkyl-bonded stationary phase and a mobile phase containing methanol or acetonitrile could be provide selectivity and pH adjustment should be capable of resolving AT and related impurities [6] if the physicochemical properties of AT and related compounds were considered. Methanol–water and acetonitrile–water at various ratios were tested as starting solvent and a considerable difference between the separation activity of methanol and acetonitrile was not observed. A clear separation could not be obtained around the peak of AT because one of unknown impurity (UNK I), DFAT and one of tablet excipient were eluted together and, the peaks of DSAT and another unknown impurity (UNK II) were not resolved from the peak of AT. Therefore, the buffer systems were tested to obtain a satisfactory resolution. For this purpose, acetate, citrate and phosphate buffer systems with the pH values between 3.0 and 5.0 were carried out since AT is an acidic compound. The mixtures of methanol–

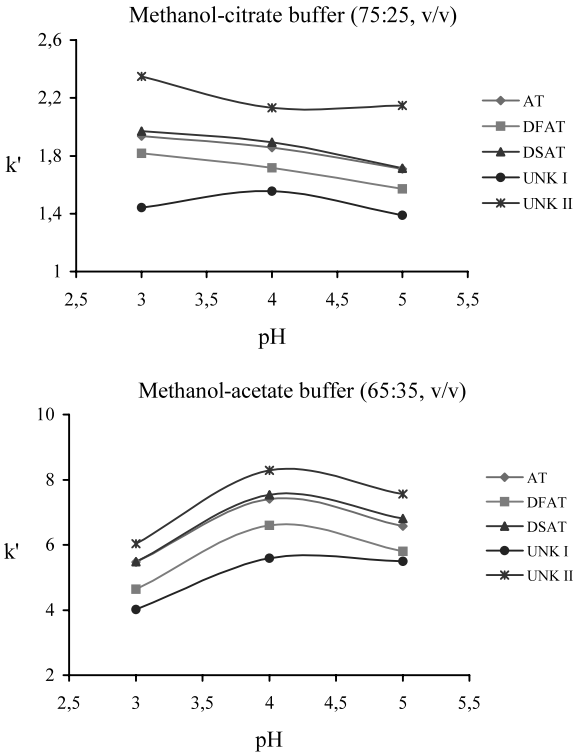


Fig. 2. Effect of citrate and acetate buffer systems and pH ranges on the capacity factors (k′) of the substances.

buffer systems at 75:25, 70:30, 65:35, 60:40 and 50:50 ratios were examined. Better results were obtained using methanol–citrate and methanol–acetate solvent systems at the ratios 75:25 and 65:35, respectively, while the methanol–phosphate buffer system did not give useful k′ values. Fig. 2 shows the effect of acetate and citrate buffer systems and pH ranges on the capacity factors (k′) of the substances at the critical separation area of the chromatogram. The best resolution was achieved at pH 4 using both mobile phase systems. On the other hand, since the peak areas obtained using acetate buffer were two times more than those of citrate buffer, further experiments were carried on with acetate buffer system although AT and DSAT still were not well resolved.

THF was tested to increase resolution of AT and DSAT. The effect of THF on the k′ values of AT and related compounds was studied at 5–20% concentration in the mobile phase, methanol–acetate buffer pH 4 (65:35, v/v). The best result

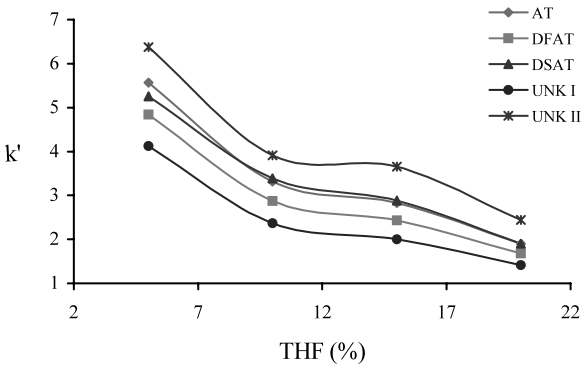


Fig. 3. Effect of THF concentration in mobile phase on the k′ values of AT and related compounds.

was obtained by adding of 5% THF in the mobile phase but the separation of AT and DSAT were not very well resolved (Fig. 3). Thus, it was considered that the gradient elution was necessary for an achieved resolution. Both methanol and acetonitrile was tested at various gradient conditions and better results were obtained using acetonitrile.

The compounds studied were resolved from AT and each other at the conditions described in experimental part. However, after several injections, the resolution was destroyed and band broading was observed. To solve this problem it was established that the column must be conditioned after each run for 60 min using the mobile phase at ending gradient conditions. So well resolved peaks that is, the reproducible results were obtained after all injections. Present HPLC method offers well resolution within 60 min. A total chromatogram of bulk drug and enlarged chromatograms of critical separation part of the tablets containing 10 and 20 mg AT were given in Figs. 4 and 5, respectively. Developed method is capable of determining the amount and purity of the active gradient and the percent level of impurities with a total chromatographic purity in a single step.

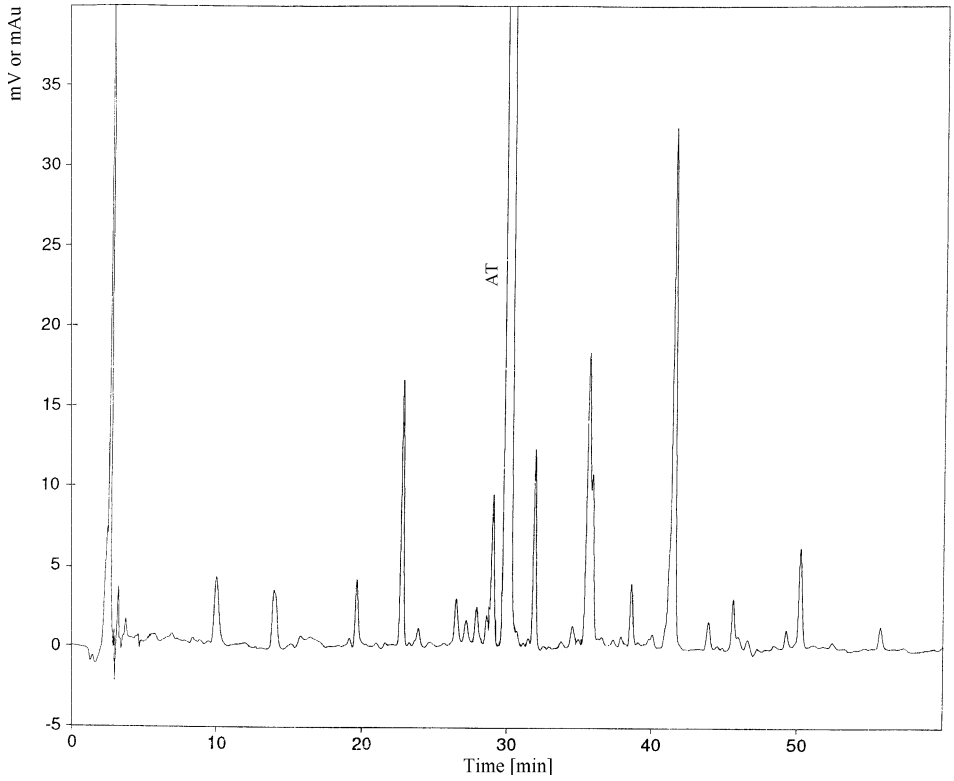


Fig. 4. The representative chromatogram obtained analysis of bulk drug.

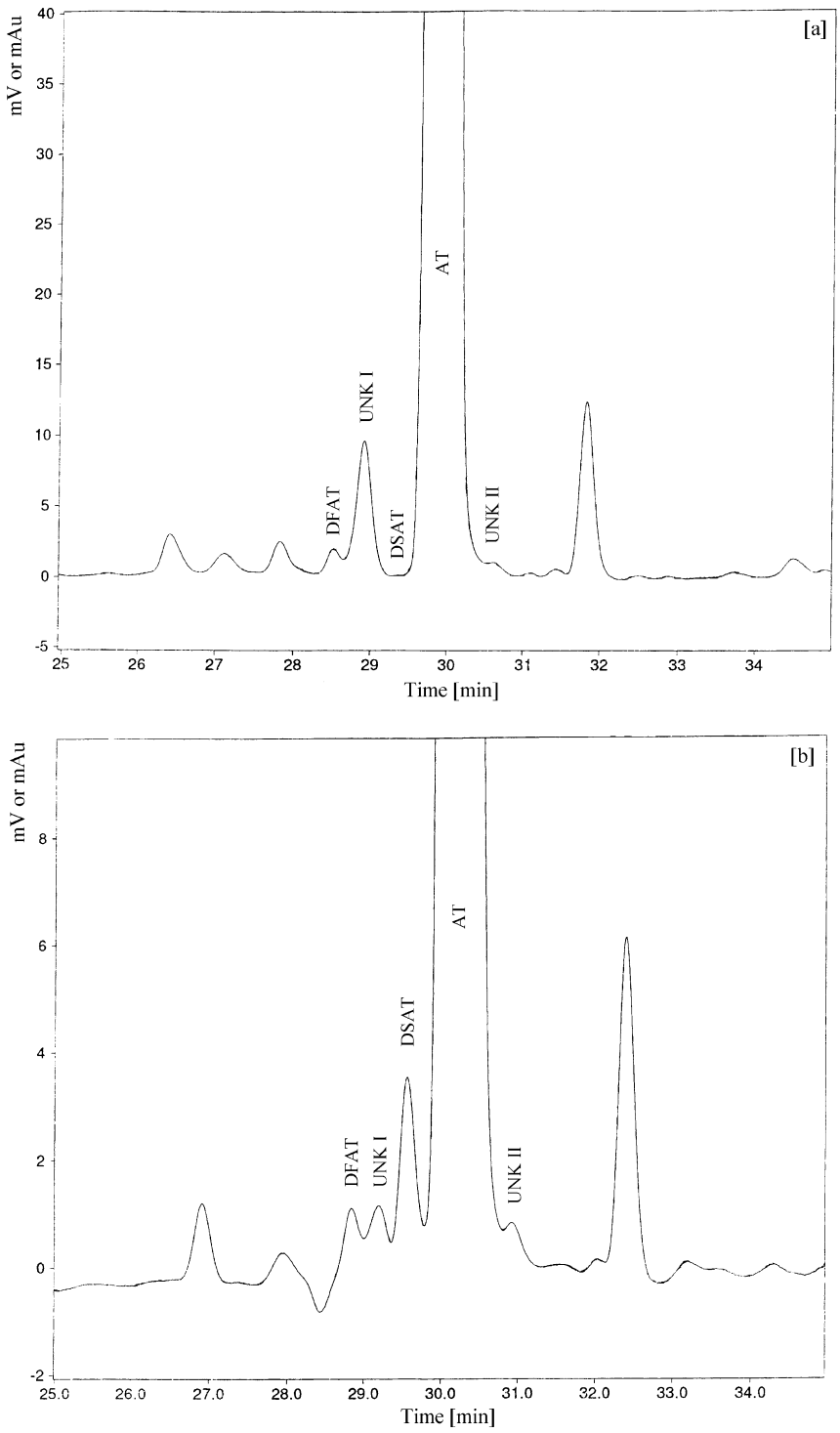


Fig. 5. Critical separation area of enlarged chromatograms obtained from analysis of Ator® 10 mg (a) and Ator® 20 mg (b) tablets.

Table 1
Validation parameters of the proposed method (n = 6)

Sample	Added (μg ml^{-1})	Found (mean ± S.D.[a])	R.S.D.[b]%	Recovery%
AT	1.00	0.99 ± 0.005	0.50	99.08
DFAT	0.10	0.09 ± 0.002	1.60	99.58
DSAT	0.10	0.09 ± 0.002	1.52	98.66

[a] Standard deviation.
[b] Relative standard deviation.

Table 2
Limit values of AT and related compounds (n = 3)

Sample	LOQ[a] (μg ml^{-1}) (mean ± S.D.)	LOD[b] (μg ml^{-1})
AT	0.130 ± 0.010	0.013
DFAT	0.050 ± 0.005	0.010
DSAT	0.050 ± 0.008	0.010

[a] Limit of quantification.
[b] Limit of detection.

The linearity of the method was studied over the range of 0.5–35.0 μg ml^{-1} for AT and 0.3–1.2 μg ml^{-1} for DFAT and DSAT. The regression equations were:

$$y = 308\ 026x - 4173.8 \quad r = 0.9999 \text{ for AT}$$

$$y = 251\ 300x + 110.31 \quad r = 0.9996 \text{ for DFAT}$$

$$y = 242\ 452x - 1421.9 \quad r = 0.9999 \text{ for DSAT}$$

The validation parameters of HPLC method were studied by using 1 μg ml^{-1} of AT and 0.1 μg ml^{-1} of DFAT and DSAT working standard solutions. The values of relative standard deviation (R.S.D.%) and recovery% of AT, DFAT and DSAT were given Table 1. Limit of detection (LOD) values were determined with a signal to noise ratio 3. Limit of quantification (LOQ) values were calculated ten times of LOD for AT and five times of LOD for DFAT and DSAT (Table 2).

Limit of each impurity and total impurity percents of AT in bulk drug should be below 0.5 and 1.5%, respectively. The developed HPLC method was applied to the percent determination of AT, DFAT, DSAT and unknown impurities in bulk drug and the tablets containing 10 and 20 mg AT. The percents of DFAT, DSAT, unknown and total impurities in the samples were performed by normalization method. The results were calculated as 0.057–0.081, 0.072–0.097 and 0.608–0.664% for DFAT, DSAT and total impurity, respectively (Table 3). Total and each impurity of all samples were determined below the limit value, as 1.5 and 0.5%, respectively.

4. Conclusion

The proposed HPLC method perfectly resolves AT, DFAT, DSAT, unknown impurities and formulation excipients of bulk drug and the tablets. The method is a simple, reliable assay for impurity determination and stability testing, which

Table 3
Assay results of bulk drug and Ator® 10 mg and Ator® 20 mg tablets (n = 6)

Sample	AT% ± S.D.[a]	DFAT% ± S.D.	DSAT% ± S.D.	TUI[a]% ± S.D.	TI[b]% ± S.D.
Bulk drug	99.60 ± 0.14	0.057 ± 0.02	0.072 ± 0.06	0.383 ± 0.08	0.608 ± 0.18
Ator® 10 mg	99.34 ± 0.18	0.069 ± 0.03	0.097 ± 0.09	0.425 ± 0.09	0.664 ± 0.18
Ator® 20 mg	99.40 ± 0.21	0.081 ± 0.04	0.086 ± 0.09	0.414 ± 0.10	0.653 ± 0.20

[a] Total unknown impurities.
[b] Total impurity.

would provide acceptable linearity, accuracy, precision and selectivity.

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References

[1] A.P. Lea, D. McTavish, Drugs 53 (1997) 828–847.
[2] E.L. Posvar, L.L. Radulovic, D.D. Cilla, L.R. Whitfield, A.J. Sedman, J. Clin. Pharmacol. 36 (1996) 728–731.
[3] X. Miao, C.D. Metcalfe, J. Chromatogr. A 998 (2003) 133–141.
[4] X. Miao, C.D. Metcalfe, J. Mass Spectrom. 38 (2003) 27–34.
[5] M. Jemal, Z. Ouyang, B. Chen, D. Teitz, Rapid Commun. Mass Spectrom. 13 (1999) 1003–1015.
[6] M. Gazdag, G. Szepesi, E. Szeleczki, J. Chromatogr. 454 (1988) 83–94.