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ORIGINAL RESEARCH

Validated analysis of fluvastatin in a pharmaceutical capsule formulation and serum by capillary electrophoresis

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ABSTRACT: The capillary electrophoretic behavior and the determination of fluvastatin (FLU) in capsule and serum is described in this study. Method development was conducted in a fused-silica capillary ($L = 86$ cm, $L_{eff} = 58$ cm and 75 μm i.d.) and a background electrolyte consisting of 10 mM borate at pH 8 was used. The separation was performed by current-controlled system applying 41 μA, detecting at 239 nm and injecting 0.5 s vacuum injection. A good electropherogram and excellent repeatability was obtained. FLU and phenobarbital sodium (internal standard) migrated (with RSD%) at 4.8 (0.3) min and 5.2 (0.6) min, respectively. Limit of detection (LOD) and limit of quantitation (LOQ) values were found to be 1×10^{-6} M and 2.89×10^{-6} M, respectively. Linearity in the range of 1.03×10^{-5} –5.15×10^{-5} M was examined employing intra-day and inter-day studies and well-correlated calibration equations were obtained. FLU in a capsule (Lescol® 40 mg declared) was found to be 41.9 ± 0.4 mg. Furthermore, FLU was determined in serum applying standard addition technique. Good repeatability and no interference were observed. The method proposed is simple, sensitive, precise and easy to use for the determination of FLU in capsule and serum. Copyright © 2001 John Wiley & Sons, Ltd.

INTRODUCTION

Fluvastatin sodium ([R*,S*,-(E)-] (±)-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2yl]-3,5-dihydroxy-6-heptenoic acid, monosodium salt), (FLU, Fig. 1) is a potent inhibitor of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase which converts HMG-CoA to mevalonate which is an early rate-limiting step in cholestrol biosyntesis (Langtry and Markham, 1999).

It has been reported that FLU shows a high rate of absorption (unaffected by food), rapid hepatic uptake (systemic exposure time is only 30 min), high protein binding (>95 %) and high hepatic excretion (95% of a dose is secreted in the bile). In clinical trials with hyperlipidemic patients, FLU produced a dose-dependent reduction in LDL cholesterol. The drug also reduced triglycerides and increased HDL cholesterol. FLU is metabolized in the liver, primarily via hydroxylation of the indole ring at the 5- and 6-positions. N-dealkylation and β-oxidation of the side chain also occurs. The hydroxy metabolites have same pharmacologic activity, but do not circulate in the blood (Langtry and Markham, 1999).

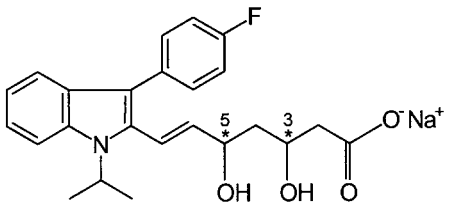

Figure 1. The chemical structure of fluvastatin.

There are several methods for the analysis of fluvastatin in human plasma by HPLC and fluorometric detection utilizing internal standard (Kalafsky *et al.*, 1993; Toreson and Eriksson, 1996, 1997). However, no capillary electrophoretic study has been reported for fluvastatin.

This paper describes a capillary zone electrophoretic method for the determination of FLU. The optimum capillary electrophoretic and analytical parameters were investigated and the metod validation studies were performed. The proposed method has been applied to the analysis of pharmaceutical capsule forumlation and human serum.

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Abbreviations used: FLU, fluvastatin; HMG-CoA, hydroxymethylglutaryl coenzyme.

EXPERIMENTAL

Apparatus. CE experiments were conducted using a Spectrophoresis 100 system equipped with Modular Injector, High Voltage Power Supply and a model Spectra FOCUS scanning CE detector (Thermo Separation Products, CA, USA) cabled to a Model Etacomp 486 DX 4-100 computer processing the data using PC 1000 (Version 2.6) working under OS/2 Warp program (Version 3.0). The analysis was performed with a fused silica capillary having a total length of 88 cm, effective length 58 cm and 75 μm i.d. (Phenomenex, Torrence, CA, USA). The pH of the solutions was measured using a model Multiline P4 pH meter with a model SenTix glass electrode (WTW, Weinheim, Germany). All the solutions used during the experiments were filtered from a model Phenex microfilter (25 mm, 0.45 μm; Phenomenex, Torrence, CA, USA) and degassed using a model B-220 ultrasonic bath (Branson, CT, USA).

Chemicals. Fluvastatin and its pharmaceutical preparation Lescol® Capsule (containing 40 mg active material) were generously provided by Novartis Sağlık, Gıda ve Tarım Ürünleri A.Ş., 80640 Istanbul, Turkey. Acetonitrile (HPLC grade), hydrochloric acid, sodium hydroxide, borax, phenobarbital sodium (internal standard, IS) were supplied by Merck (Darmstadt, Germany). Double-distilled water was used throughout this study.

Procedure for CE instrumentation. Fused silica capillary tubing was filled with the background electrolyte (pH 8; 10 mM borate). Both ends of the tube were dipped into reservoir (8 mL) and vial (1.8 mL) filled with background buffer. The end part in which the sample (side of vial) introduced was connected with a platinum electrode positive high voltage side of the power supply. The reservoir side at the detector end was connected with a platinum electrode to ground. Samples at a concentration 3.09×10^{-5} M for the optimization of CE parameters were introduced by 0.5 s of vacuum injection corresponding to almost 25 nL.

Washing program was applied before each run. Silica capillary was purged for 2.5 min with 0.1 M sodium hydroxide solution followed by 2.5 min double-distilled water. Then, it was equilibrated passing through the background electrolyte for 2.5 min prior to analysis.

Procedures. A stock solution of FLU was prepared by weighing about 21 mg of FLU dissolved in 50 mL of distilled water. Five dilutions were made in the range of 1.03×10^{-5} and 5.15×10^{-5} M, each containing 1 μmol IS. All the dilutions were prepared in the background electrolyte of 10 mM borate buffer at pH 8.

Current-controlled analysis was employed keeping the current fixed at 41 μA (about 28 kV in our system). The sample was injected 0.5 s and detected at 239 nm.

Analysis of FLU capsules. Ten FLU in capsules (Lescol® capsules) were accurately weighed. The average weight of one capsule was calculated and then they were finely mixed in a mortar. A sufficient amount of capsule powder equivalent to 20 mg of FLU was accurately weighed, transferred to a 50 mL flask and made up to volume by distilled water. It was magnetically stirred for 10 min and made up to the final volume with related solvent. The solution was then centrifuged at 5000*g* for 10 min. The supernatant and fix amount of IS solutions were diluted with background electrolyte and it was injected to the CE.

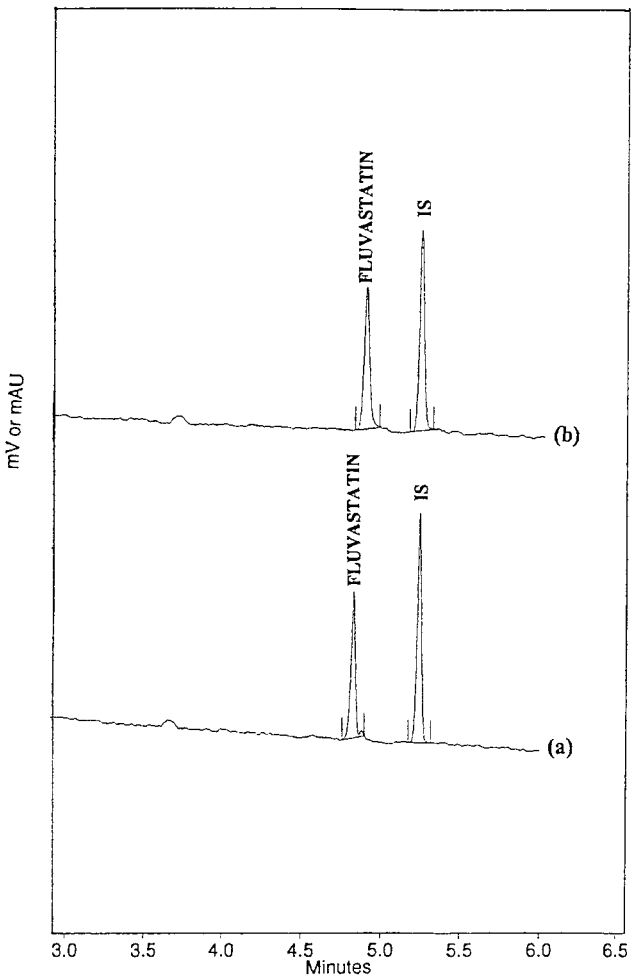

Figure 2. The electropherograms of FLU and IS (phenobarbital sodium). Conditions: running buffer 10 mM borate (pH 8.0); current controlled used, 41 μA; injection time, 0.5 s hydrodynamically; detection wavelength, 239 nm; capillary total length 86 cm with an effective length of 58 cm to the detector and 75 μm i.d. (a) In the standard solution (FLU, 3.09×10^{-5} M; IS, 1 μmol); (b) in the capsule solution (IS, 1 μmol).

Analysis of FLU in serum. Blood samples were withdrawn from healthy volunteers (consents were obtained from all patients and approved by the Ethics Committee) and the serum samples were separated by centrifuging for 10 min at 5000*g*.

A certain concentration of FLU was transferred into 1 mL serum and the contents of the tube were vigorously shaken. Then, 3 mL acetonitrile containing 6×10^{-5} phenobarbital sodium (IS) was added and mixed well using a shaker. The precipitated proteins were separated by centrifuging for 10 min at 5000*g*. The clear solution was directly injected to CE under the same conditions as described earlier.

Table 1. Precision of migration time, peak area, peak normalization and their ratio values of 1.03×10^{-5} M of FLU and 1 μmol IS

	Mean (RSD%) values			
	Migration time	Peak area	Peak normalization (PN)	PN_{FLU}/PN_{IS}
FLU	4.9 (0.3)	1005 (3.4)	205.1 (3.0)	0.245 (2.2)
IS (phenobarbital sodium)	5.2 (0.6)	4396 (1.1)	895.4 (0.9)	

The data presented are averages of eight experiments.

Table 2. Some capillary electrophoretic parameters including FLU and IS

	FLU	IS
Mobility ($cm^2\ V^{-1}\ s^{-1}$)	2.2×10^{-4}	2.6×10^{-4}
Capacity factor	8.6555	9.4898
Peak width at base (min)	0.052	0.049
Plates	139699	179885
Resolution	8.25	8.89

Table 3. Linearity and accuracy of the method

Parameters	Intra-day precision ($k = 1$; $n = 5$)	Inter-day precision ($k = 3$; $n = 15$)
Slope ± SD	25437 ± 36	25331 ± 1980
Intercepts	−0.028	−0.027
Correlation coefficient (r)	0.9998	0.9996
Slope ± CL (p = 0.05)	25437 ± 34	25331 ± 900

k, number of sets; *n*, number of standards.

RESULTS AND DISCUSSION

Certain attempts were performed to find out the suitable composition of the background electrolyte and optimum instrumental parameters for the determination of FLU.

Since FLU has a carboxylic group, a slightly basic solution could be suitable for the separation system. Various type of background electrolyte have been used for the analysis of carboxylic acids (Kitagishi and Shintani, 1998). The best suitable background electrolyte consisted of 10 mM borate at pH 8. The optimum instrumental conditions were investigated by varying the related parameters. FLU was detected at 239 nm. The injection time of 0.5 s was used because higher duration caused zone broadening. Current-controlled experiments were achieved adjusting the current to 41 μA. FLU and phenobarbital sodium (IS) migrated in a very reasonable time. A typical electropherogram of FLU and IS is shown in Fig. 2(a).

FLU and IS showed well-shaped peaks with a symmetry factor of 0.80 and 0.86, respectively. Furthermore, the peaks did not interfere with any other signals from the excipients of the capsule or the endogenous substances in the serum. Therefore, the proposed method is selective with a selectivity degree ($\alpha = 8.25$).

The repeatability of the system was examined by injecting 1.03×10^{-5} M of FLU and 1 μmol IS with eight successive injections and they were evaluated considering migration time, area, peak normalization and ratio of the peak normalization values of FLU and IS. The precision values with their RSD% are shown in Table 1.

When compared, better results are obtained by using the ratio values. Some capillary electrophoretic parameters were calculated from the electropherogram as shown in Table 2. The limit of detection (LOD) at S/N = 3.3 and limit of quantitation (LOQ) at S/N = 10 were found to be 1.0×10^{-6} and 2.89×10^{-6}, respectively.

Linearity

Linearity was investigated in the $1.03 \times 10^{-5} - 5.15 \times 10^{-5}$ M range as three sets. The results are shown in Table 3 and they indicate accuracy for intra or inter-day experiments with a good correlation. The results indicate that the method could be used confidently for the analysis of FLU in pharmaceutical preparations and body fluids such as serum and urine.

Analysis of the pharmaceutical capsules

The determination procedure of FLU in capsules was followed as given in the Experimental section. Analysis was performed under optimum conditions. Eight independent experiments were performed and the content of the capsules was determined. No interference was observed originating from inactive ingredients or excipients. The electropherogram of pharmaceutical capsule solution of FLU is shown in Figure 2(b). The results were statistically evaluated as demonstrated in Table 4.

Repeatability of the eight experiments was found to be 1.1%, indicating the precision of the analysis. The amount of the active material was found to be 4.75% higher than the declared amount, which is still within the

Table 4. The results of capsule assay (Lescol® Capsule contains 40 mg active material)

Mean (mg)	41.9
Standard deviation (SD)	1.2
Relative standard deviation (RSD%)	1.1
Confidence limits (CL)	±0.4

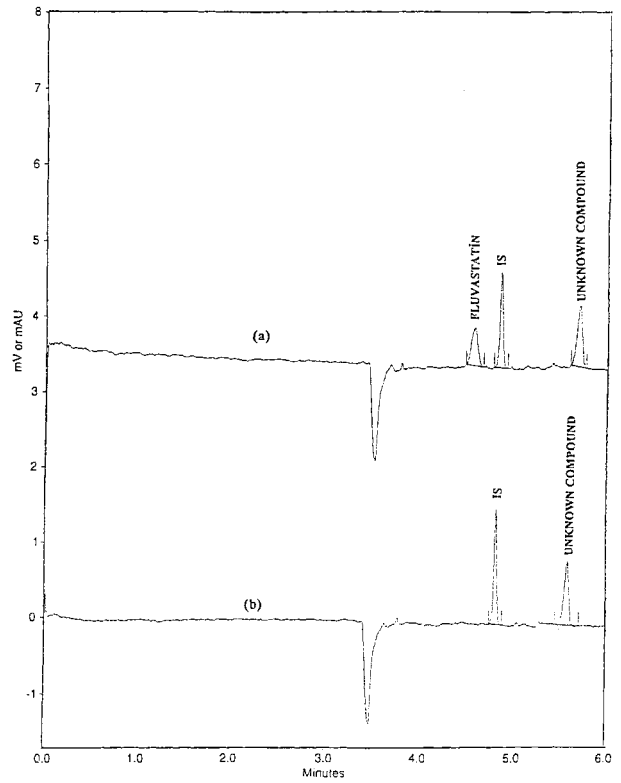

Figure 3. The electropherograms of FLU and IS (phenobarbital sodium), conditions are the same as in Figure 2. (a) In the serum solution spiked with fluvastatin (FLU, 2.5×10^{-5} M; IS 1.88×10^{-5}); (b) in the blank serum (IS, 1.88×10^{-5}).

acceptable deviation range of ±5% of the declared amount.

Analysis of FLU in human serum

Analysis of drugs from serum by CE does not usually require extensive time-consuming sample preparation, use of expensive organic solvents and other chemicals. The serum proteins are precipitated by the addition of acetonitrile (1 part serum:3 parts acetonitrile) which is centrifuged at 5000***g*** and the supernatant is directly injected and analyzed.

Table 5. The results of serum application for recovery studies

Final concentration of FLU	Recovery for FLU (%)
6.25×10^{-6}	86.6
1.25×10^{-5}	92.0
2.5×10^{-5}	91.4
7.75×10^{-5}	94.7
5.0×10^{-5}	95.3

Figures 3 shows the electropherograms obtained from the serum spiked with FLU and IS and blank serum, respectively, which indicate no interferences from the endogenous substances present in the serum. The determination of FLU in serum was investigated by applying a standard method applied at five dilutions. The recoveries are shown in Table 5.

A linear dependence of the FLU concentrations were exhibited for the sets with or without serum. The recovery of FLU was calculated as FLU serum/$FLU_s \times 100$ where FLU is the concentration of fluvastatin measured in serum and FLU_s is the concentration of fluvastatin in the background buffer solution. Since the proteins are removed by precipitation, the bounded material normally decreases the recovery percentage. The average amount of unbounded FLU is 92%. It has already been reported that the unbounded FLU was about 90%, indicating that the technique which is used here can be employed to elucidate certain parameters related to the protein binding.

In conclusion, the method proposed here is sensitive, precise and easy to use for the determination of FLU in capsule and serum. Therefore, it can be used for the quality control laboratories and also in bioavailability and pharmacokinetic studies of the drug.

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